Egg production and quality responses to increasing isoleucine supplementation in Shaver white hens fed a low crude protein corn-soybean meal diet fortified with synthetic amino acids between 20 and 46 weeks of age

Ilona A. Parenteau,* Marvin Stevenson,† and Elijah G. Kiarie*,[1]

*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada; and †Halchemix, Port Perry, ON, Canada

ABSTRACT The present study evaluated production performance responses to Ile supplementation in laying hens fed low crude protein (**LCP**), amino acid (**AA**) balanced diets. A total of 179 Shaver white pullets were distributed into 30 battery cages (6 birds/cage, n = 6) and observed over the course of 27 wk in a 2-phase (20 to 27 and 28 to 46 wk of age) feeding program. Five isocaloric diets were formulated for standardized ileal digestible (**SID**) Lys intake of 750 and 710 mg/D in phase 1 and 2, respectively, and included a positive control with standard levels of crude protein (**CP**) (**CON**; 18 and 16% CP for phases 1 and 2), and 4 LCP diets (16 and 14% CP for phase 1 and 2, respectively) with graded levels of Ile to satisfy SID Ile:Lys ratios of 70 (**Ile70**), 80 (**Ile80**), 90 (**Ile90**), and 100% (**Ile100**). Based on analyzed dietary AA, the calculated SID Ile:Lys of LCP diets were 75, 84, 88, 99% and 66, 72, 82, 95% for phase 1 and 2, respectively. Dietary treatments significantly ($P < 0.05$) affected feed intake, hen-day egg production (**HDEP**), egg weight (**EW**), feed conversion ratio, and egg quality (Haugh unit) and composition (yolk to albumen). Lowering dietary CP negatively affected HDEP with a 3.3 and 1.5% reduction in phase 1 and 2, respectively, and this was restored with the addition of Ile ($P < 0.001$) suggesting that Ile was limiting in the LCP basal diet. Average EW was reduced in Ile100 only; however, the Ile:Lys appeared to influence egg size uniformity, with Ile90 producing a greater proportion of large ($56 \text{ g} \leq \text{EW} > 63 \text{ g}$) eggs, suggesting that Ile may be used to manipulate EW at the expense of HDEP. Overall, the results indicated that CP in laying hen diets can be reduced by 2% units if fortified with synthetic AA (Met, Lys, Thr, Trp) + Ile, with optimal responses observed between 82 and 88% SID Ile:Lys.

Key words: laying hen, isoleucine, low crude protein, egg production and quality

2020 Poultry Science 99:1444–1453
https://doi.org/10.1016/j.psj.2019.10.064

INTRODUCTION

Consumer demand for eggs in North America has been steadily increasing, with retail growth of 6% in 2018 in Canada, up from the 4.1% growth seen in 2017 (Egg Farmers of Canada, 2017, 2018). In the United States, 2018 per capita egg consumption increased by 2.1% over 2017 (USDA, 2019). It is therefore critical to establish suitable nutritional and management strategies that accommodate these increased production goals, while accounting for the economic, social, and environmental challenges associated with modern egg production. The conventional use of commercially manufactured amino acids (**AA**) is primarily to provide nutritionists with the flexibility to counter fluctuations in commodity pricing; however, secondary benefits of applying the ideal protein concept to formulations include mitigating N losses and improving hen health and welfare (Summers, 1993; Kristensen and Wathes, 2000; Burley et al., 2013). As dietary crude protein (**CP**) is reduced, special attention must be paid to the level of essential AA (**EAA**) contributed by the diet, as an imbalance of AA will result in compromised performance (Keshavarz and Jackson, 1992; Meluzzi et al., 2001; Bregendahl et al., 2008).

Commercially available crystalline AA include Met, Lys, Thr, Trp, and Val; therefore, most research has focused on these AA. Recent commercialization of Ile has prompted further interest in optimizing low crude protein (**LCP**) diets for laying hens. Evidence indicates

Received August 12, 2019.
Accepted October 24, 2019.
Presented in part at the Poultry Science Association 2018 annual meeting, San Antonio TX, July 23-26.

[1]Corresponding author: ekiarie@uoguelph.ca

that Ile is a limiting AA in corn- and SBM-based diets for laying hens with reduced CP content (Bray, 1964; Harms and Russell, 2000; Shivazad et al., 2002; Peganova et al., 2003), and several studies reported increased rate of lay (Jensen, 1991; Harms and Russel, 1993; Sohail et al., 2002; Ospina Rojas et al., 2015) and egg weight (Morris and Gous, 1988; Keshavarz and Jackson, 1992; Harms and Russel, 1993; Sohail et al., 2002) with Ile supplementation. However, estimates for Ile requirements for laying hens are inconsistent and range from 400 to 780 mg/D. Much of these data are reported on a total intake basis, which does not account for digestibility differences between ingredients nor the influence of environmental factors on absolute AA requirements (Miller et al., 1954; Bray, 1969; Harms and Ivey, 1993; NRC, 1994; Coon and Zhang, 1999; Harms and Russell, 2000; Shivazad et al., 2002).

Few experiments have been conducted on standardized ileal digestible (**SID**) Ile:Lys requirements in broiler chicks (Baker et al., 2002; Corzo et al., 2009; Berres et al., 2010) and broiler breeders (de Lima et al., 2016, 2018); however, there are limited experimental data for modern laying hens. Bregendahl et al. (2008) conducted 7 simultaneous experiments to determine the ideal AA profile for layers and estimated the ideal SID Ile:Lys to be 79%, which agrees with previous reports (CVB, 1996; Leeson and Summers, 2005). These recommendations, however, are outdated and need to be reevaluated as the genetic progression of layer strains has led to a tremendous increase in egg mass (**EM**) output and feed efficiency (Elliot, 2008). Modern laying hens enter lay at a younger age, exhibit much higher rate of lay throughout the production period, and have a lower body weight (**BW**) compared to older strains (Elliot, 2008; Bain et al., 2016). As such, AA requirements for maintenance and production have inevitably changed and will continue to change with the progression of genetics and alternative management systems. Reports on AA requirements for the modern laying hen are limited and inconsistent, and this is further complicated by a lack of data on AA bioavailability among different ingredients, particularly under crude protein restriction. To develop a practical understanding of crystalline Ile use in LCP diets, a study was conducted to evaluate production performance and egg quality responses to different levels of L-Ile in laying hens fed diets reduced by 2% points of CP from a commercial standard (control treatment) in which Ile requirements were provided only through raw ingredients.

MATERIALS AND METHODS

The present study was conducted at Arkell Research Station and was approved by the University of Guelph Animal Ethics Committee and complied with the Canadian Code of Practice for the Care and Use of Animals for Scientific Purposes (CCAC, 2009).

Birds and Housing

A total of 179 White Shaver laying hens aged 19 wk were obtained from Arkell Poultry Research Station (University of Guelph, Guelph, Canada) flocks. Hens were placed in 30 battery cages (6 birds/cage) based on BW and allocated to 5 experimental diets in a completely randomized design (6 replicates per diet) so that cage BW were uniform (CV $< 4\%$) across treatments ($1.39 \text{ kg} \pm 0.06$). Birds were adapted to diets for 1 wk before data collection began, and the experimental period was divided into a 2-phase feeding program (phase 1: 20 to 27 wk of age, phase 2: 28 to 46 wk of age). Owing to a shortage of hens, one cage in Ile100 contained only 5 hens at trial initiation; during phase 2, one of the hens in this cage was found sick and was euthanized. At 29 wk of age, one bird/cage was necropsied for samples not reported herein.

Hens were fed and managed in accordance with the White Shaver Commercial Management Guide (Hendrix ISA, 2011) and were housed in a windowless, fan-ventilated, light- and temperature-controlled room (14 h at 60% intensity, 20°C). Cages (H49.5 × W62.2 × D66.0 cm) were equipped with one nipple drinker and an exterior feed trough that expanded the length of the cage. Feeders were fitted with a wire mesh as previously described by Mwaniki et al. (2018) to minimize feed wastage but not to restrict feed consumption. Feed and water were provided *ad libitum* during the entire experimental period.

Dietary Treatments

Five isocaloric diets were formulated on the ideal protein concept and were designed to deliver a SID Lys intake of 750 and 710 mg/D during phase 1 and 2, respectively, which are approximately 10% below breeder-recommended levels to ensure Lys was second limiting in the protein mixture. Ingredient AA concentrations were from Ajinomoto Animal Nutrition North America (Chicago) and digestibility coefficients were obtained from Evonik AminoDat database (Essen, Germany). A control diet (**CON**) with a calculated CP level of 18% and SID Ile:Lys ratio of 80% was provided and compared to 4 low crude protein (**LCP**; 16% CP) diets with graded levels of crystalline L-isoleucine (Table 1). For phase 2 diets, CP was reduced to 16 and 14% for CON and LCP diets, respectively, and all other nutrient levels were adjusted accordingly to meet estimated requirements as per the breeder manual (Table 2). Basal LCP diets were formulated to contain 0.52 and 0.50% SID Ile for phases 1 and 2, respectively, and crystalline Ile was added at the expense of cornstarch to meet SID Ile:Lys ratios of 70 (**Ile70**), 80 (**Ile80**), 90 (**Ile90**), and 100% (**Ile100**). For phase 1, 2 mixtures with the highest and lowest levels of Ile were blended to create the other 2 diets. Owing to the limited mixer capacity at the facility, all phase 2 diets were mixed in 5 separate 600 kg batches. Complete diets were prepared in crumble form at the Arkell Research

Table 1. Composition and calculated nutrient levels of phase 1 (20 to 27 wk of age) diets.[1]

Item	CON	Ile70	Ile80	Ile90	Ile100
Ingredient (g/kg)					
Corn	485.1	476.9	476.9	476.9	476.9
SBM 46%	177.4	139.3	139.3	139.3	139.3
Wheat	100.0	150.0	150.0	150.0	150.0
Limestone fine	66.3	70.0	70.0	70.0	70.0
Limestone course	19.9	21.0	21.0	21.0	21.0
Pork meal-58%	60.0	42.0	42.0	42.0	42.0
Corn DDGS	40.0	40.0	40.0	40.0	40.0
Corn starch	15.00	15.00	14.20	13.37	12.57
Soy oil	11.9	10.8	10.8	10.8	10.8
Vitamin and trace premix[2]	10.0	10.0	10.0	10.0	10.0
Monocalcium phosphate	5.1	11.3	11.3	11.3	11.3
Sodium bicarbonate	1.0	2.5	2.5	2.5	2.5
Salt	2.3	1.5	1.5	1.5	1.5
DL-Methionine-99%	2.13	2.57	2.57	2.57	2.57
L-Lysine HCL	0.73	2.30	2.30	2.30	2.30
L-Threonine-98.5%	0.27	0.97	0.97	0.97	0.97
L-Valine-95%	0.00	0.60	0.60	0.60	0.60
L-Tryptophan-98.5%	0.00	0.17	0.17	0.17	0.17
L-Isoleucine-92%	0.00	0.07	0.90	1.70	2.50
Titanium dioxide	3.00	3.00	3.00	3.00	3.00
Calculated provisions					
AMEn (kcal/kg)	2,800	2,800	2,800	2,800	2,800
Crude protein (%)	18	16	16	16	16
SID amino acids (%)					
Arg	0.98	0.83	0.83	0.83	0.83
Glu	2.84	2.70	2.70	2.70	2.70
His	0.38	0.33	0.33	0.33	0.33
Ile	0.60	0.52	0.60	0.67	0.75
Leu	1.33	1.19	1.19	1.19	1.19
Leu + Val	1.76	1.67	1.67	1.67	1.67
Lys	0.75	0.75	0.75	0.75	0.75
Met	0.46	0.48	0.48	0.48	0.48
Met + Cys	0.67	0.68	0.68	0.68	0.68
Phe	0.70	0.61	0.61	0.61	0.61
Phe + Tyr	1.14	1.01	1.01	1.01	1.01
Thr	0.52	0.52	0.52	0.52	0.52
Trp	0.17	0.16	0.16	0.16	0.16
Val	0.68	0.65	0.65	0.65	0.65
Calcium (%)	4.10	4.20	4.20	4.20	4.20
Total phosphorus (%)	0.69	0.72	0.72	0.72	0.72

Abbreviations: CON, control; SID, standardized ileal digestible.

[1]CON: Control treatment formulated to provide Ile requirements through intact protein only. Ile70-100: Protein-restricted diets fortified with L-Ile to meet SID Ile:Lys ratios of 70, 80, 90, 100.

[2]Vitamins and minerals provided per kilogram of premix: vitamin A, 1,200,000 IU; vitamin D3, 500,000 IU; vitamin E, 8,000 IU; vitamin B12, 1,700 mcg; biotin, 22,000 mcg; menadione, 330 mg; thiamin, 400 mg; riboflavin, 800 mg; pantothenic acid, 2,000 mg; pyridoxine, 430 mg; niacin 65,000 mg; folic acid, 220 mg; choline, 60,000 mg; iron, 6,000 mg; copper, 1,000 mg, manganese, 7,000 mg, zinc, 7,000 mg, iodine, 100 mg.

Station feed mill (University of Guelph, Canada). The conditioning temperature was 60°C–65°C and steam pressure was 30 psi. Titanium dioxide was added for determination of nutrient digestibility (data not presented in the current report).

Experimental Procedures and Data Collection

Labeled buckets with the respective diet were randomly assigned to each cage and were filled once every 2 wk. Feed intake (**FI**) was recorded by calculating the difference between full bucket weights and remaining feed after the 2-wk period. Body weights (cage weight/number of hens per cage) were recorded at placement, at 20 wk of age, and every 4 wk thereafter until the end of the experiment. Production performance was quantified through hen-day egg production (**HDEP**), egg weight (**EW**), EM (HDEP × EW), egg quality (Haugh unit, shell strength), egg composition (yolk to albumen ratio; **Y:A**), and feed conversion ratio (**FCR**; FI/EM). Egg production was recorded each morning at 10 am, and a weekly average was used to calculate HDEP (%).

Individual eggs were weighed biweekly over 2 consecutive days using a precision scale (Sartorius Entris 6202-1S, Cole-Parmer Canada Company, Montreal, QC, Canada). Internal egg quality and shell breaking strength (kgf) of all eggs laid were evaluated once every 2 wk in phase 1, and every 4 wk in phase 2 using EggAnalyzer (ORKA Food Technology Ltd., Ramat HaSharon, Israel) and Force Reader (ORKA Food Technology Ltd.), respectively, as described by Mwaniki et al. (2018). In phase 2, egg composition of 2 randomly selected eggs per cage was also measured. For egg

Table 2. Composition and calculated nutrient levels of phase 2 (28 to 46 wk of age) diets.[1]

Item	CON	Ile70	Ile80	Ile90	Ile100
Ingredient (g/kg)					
Corn	549.5	546.0	546.0	546.0	546.0
SBM 46%	185.2	162.6	162.6	162.6	162.6
Wheat	80.0	80.0	80.0	80.0	80.0
Limestone fine	67.7	71.7	71.7	71.7	71.7
Limestone course	27.1	28.7	28.7	28.7	28.7
Pork meal-58%	30.6	10.0	10.0	10.0	10.0
Corn DDGS	5.0	5.0	5.0	5.0	5.0
Corn starch	15.00	46.34	45.59	44.82	44.04
Soy oil	5.2	5.5	5.5	5.5	5.5
Vitamin and trace premix[2]	10.0	10.0	10.0	10.0	10.0
Monocalcium phosphate	14.4	20.2	20.2	20.2	20.2
Sodium bicarbonate	1.9	2.3	2.3	2.3	2.3
Salt	2.3	2.3	2.3	2.3	2.3
DL-Methionine-99%	2.08	2.51	2.51	2.51	2.51
L-Lysine HCL	0.82	2.10	2.10	2.10	2.10
L-Threonine-98.5%	0.35	0.96	0.96	0.96	0.96
L-Valine-95%	0.00	0.72	0.72	0.72	0.72
L-Tryptophan-98.5%	0.00	0.11	0.11	0.11	0.11
L-Isoleucine-92%	0.00	0.00	0.75	1.52	2.30
Titanium dioxide	3.00	3.00	3.00	3.00	3.00
Calculated values (%)					
AMEn (kcal/kg)	2,750	2,750	2,750	2,750	2,750
Crude protein	16	14	14	14	14
SID amino acids (%)					
Arg	0.90	0.77	0.77	0.77	0.77
Glu	2.74	2.54	2.54	2.54	2.54
His	0.35	0.31	0.31	0.31	0.31
Ile	0.57	0.50	0.57	0.64	0.71
Leu	1.24	1.11	1.11	1.11	1.11
Leu + Val	1.74	1.69	1.69	1.69	1.69
Lys	0.71	0.71	0.71	0.71	0.71
Met	0.43	0.45	0.45	0.45	0.45
Met + Cys	0.64	0.64	0.64	0.64	0.64
Phe	0.66	0.58	0.58	0.58	0.58
Phe + Tyr	1.09	0.98	0.98	0.98	0.98
Thr	0.50	0.50	0.50	0.50	0.50
Trp	0.16	0.16	0.16	0.16	0.16
Val	0.63	0.62	0.62	0.62	0.62
Calcium (%)	4.3	4.4	4.4	4.4	4.4
Total phosphorus (%)	0.73	0.73	0.73	0.73	0.73

Abbreviations: CON, control; SID, standardized ileal digestible.

[1]CON: Control treatment formulated to provide Ile requirements through intact protein only. Ile70-100: Protein-restricted diets fortified with L-Ile to meet SID Ile:Lys ratios of 70, 80, 90, 100.

[2]Vitamins and minerals provided per kilogram of premix: vitamin A, 1,200,000 IU; vitamin D3, 500,000 IU; vitamin E, 8,000 IU; vitamin B12, 1,700 mcg; biotin, 22,000 mcg; menadione, 330 mg; thiamin, 400 mg; riboflavin, 800 mg; pantothenic acid, 2,000 mg; pyridoxine, 430 mg; niacin 65,000 mg; folic acid, 220 mg; choline, 60,000 mg; iron, 6,000 mg; copper, 1,000 mg, manganese, 7,000 mg, zinc, 7,000 mg, iodine, 100 mg.

contents, yolk was manually separated from the albumen, chalazae were carefully removed using a pair of forceps, and the yolk was weighed using a precision scale. Shells with membranes were washed, air-dried at ambient temperature, and weighed. Albumen weight was calculated as the difference between EW and yolk + shell weight.

Diet Analyses

Representative samples of all 5 diets for each phase were pooled, milled to pass through a 1 mm sieve, and submitted for CP and AA analyses by Ajinomoto Animal Nutrition Group (AOAC 990.03, AOAC 994.12, AOAC 999.13; Eddyville). Gross energy was determined using bomb calorimetry (IKA Calorimeter system, C5000 Duo-control) and mineral analysis (Ca, P, K, Mg, Na) was carried out using AOAC 985.01) in a commercial laboratory (SGS Laboratories, Guelph, Canada).

Statistical Analysis

For all data analyses, cage was designated as experimental unit; therefore, an average of the individual EW was calculated for performance data, and FI was determined by dividing feed consumption of each experimental unit by the number of birds within each unit.

Outlier analyses were performed using the distribution platform in JMP 13.2.1 (SAS Institute Inc., Cary, USA), and data points detected outside of the box and whisker graphs were removed from analyses. All data were separated by phase and subjected to PROC GLM in SAS Studio using a two-way ANOVA with diet and age as main effects, and treatment means were compared using

Table 3. Analyzed nutrient composition of phase 1 (20 to 27 wk of age) diets.

Item	CON	Ile70	Ile80	Ile90	Ile100
Gross energy (kcal/kg)	3,539	3,451	3,554	3,496	3,559
Crude protein (%)	18.57	17.06	17.80	17.28	17.57
SID amino acids (%)					
Arg	0.97	0.89	0.88	0.86	0.83
Cys	0.22	0.21	0.21	0.21	0.20
His	0.37	0.34	0.34	0.33	0.32
Ile	0.64	0.60	0.68	0.70	0.76
Leu	1.39	1.28	1.29	1.28	1.24
Lys	0.76	0.80	0.81	0.79	0.77
Met	0.38	0.40	0.40	0.41	0.42
Phe	0.77	0.71	0.72	0.71	0.68
Thr	0.54	0.56	0.55	0.54	0.54
Trp	0.16	0.15	0.16	0.16	0.15
Val	0.72	0.71	0.71	0.71	0.69
Met + Cys	0.58	0.58	0.58	0.59	0.59
Calcium (%)	3.75	3.79	4.04	3.79	3.59
Total phosphorus (%)	0.61	0.69	0.66	0.69	0.64

Abbreviations: CON, control; SID, standardized ileal digestible.

Tukey's multiple comparison. Owing to facility limitations, only 4 levels of Ile were evaluated which is insufficient to model requirements; however, orthogonal contrasts were performed to determine effects of CP reduction without added L-Ile (CON vs. Ile70), CP reduction with added L-Ile at the estimated requirements (CON vs. Ile80), as well as linear and quadratic effects of Ile in LCP diets. Linear and nonlinear coefficients were derived using the ORPOL function in PROC IML for dose response of Ile in LCP diets. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Analyzed AA and nutrient levels of phase 1 and 2 diets are shown in Tables 3 and 4, respectively, and both calculated and analyzed ratios of Ile to Lys on a total and SID basis are shown in Table 5. Average dietary SID Ile:Lys ratio for phases 1 and 2 were 3.1% higher and 7.4% lower, respectively, compared to the expected ratios. Although the calculated CP and AA levels were not achieved, differences existed between the diets, and thus, the effects of CP reduction and Ile supplementation could be explored.

Dietary treatment did not affect ($P > 0.05$) BW or BW gain (data not shown); however, there was a response ($P < 0.05$) in production performance to dietary treatments (Table 6). One cage from the Ile100 treatment was removed from egg production data as 78% of the HDEP data points were deemed outliers for unknown reasons; therefore, only 5 replicates were included for this treatment. Hen-day egg production decreased by 3.3 and 1.5% in phase 1 and 2, respectively, when hens received a diet with reduced CP content (CON vs. Ile70); however, this decrease was restored with the addition of crystalline Ile in LCP diets, suggesting that Ile was limiting. Several reports suggest that the major response to protein restriction is a reduction in the rate of lay, rather than EW which tends to be more sensitive to AA imbalance (Morris and Gous, 1988; Huyghebaert et al., 1991; Meluzzi et al., 2001; Casartelli et al., 2005; Roberts et al., 2007). According to the NRC (1994), the most common dietary method of controlling egg size in older hens is restricting intake of certain EAA such as Met and Ile; however,

Table 4. Analyzed nutrient composition of phase 2 (28 to 46 wk of age) diets.

Item	CON	Ile70	Ile80	Ile90	Ile100
Gross energy (kcal/kg)	3,616	3,437	3,374	3,424	3,404
Crude protein (%)	17.0	15.6	15.0	15.0	14.9
SID amino acids (%)					
Arg	0.94	0.83	0.79	0.81	0.79
Cys	0.21	0.19	0.19	0.19	0.19
His	0.36	0.32	0.31	0.32	0.31
Ile	0.58	0.52	0.54	0.63	0.72
Leu	1.29	1.19	1.14	1.19	1.17
Lys	0.78	0.78	0.75	0.77	0.76
Met	0.40	0.39	0.38	0.39	0.39
Phe	0.76	0.69	0.66	0.67	0.66
Thr	0.53	0.51	0.49	0.53	0.51
Trp	0.17	0.15	0.15	0.15	0.15
Val	0.64	0.61	0.59	0.63	0.65
Met + Cys	0.57	0.56	0.54	0.55	0.55
Calcium (%)	3.55	3.92	4.28	4.06	3.78
Total phosphorus (%)	0.61	0.67	0.68	0.69	0.63

Abbreviations: CON, control; SID, standardized ileal digestible.

Table 5. Calculated and analyzed Ile:Lys ratios of dietary treatments.

	Diet Ile:Lys (%)					
	Calculated		Analyzed total		Analyzed SID[1]	
Diet	Total	SID[1]	Phase 1[2]	Phase 2[3]	Phase 1	Phase 2
CON	78	80	83	72	85	74
Ile70	68	70	74	66	75	66
Ile80	78	80	83	71	84	72
Ile90	88	90	86	80	88	82
Ile100	98	100	95	92	99	95

Abbreviations: CON, control; SID, standardized ileal digestible.
[1]Calculated using digestibility coefficients from broiler chick assay.
[2]Fed from 20 to 27 wk of age.
[3]Fed from 28 to 46 wk of age.

proportional decreases in egg production often accompany reduced EAA intake. A large-scale commercial evaluation on Ile indicated that EW was affected before HDEP when Ile became limiting (Elliot, 2008). In the present study, all treatments maintained a similar EW ($P > 0.05$) except for Ile100, which yielded the smallest eggs in both phases. Thus, it appears that an excess intake of Ile limited egg size while maintaining HDEP at the same level of the control, whereas the opposite is true for limited Ile intake in the Ile70 treatment. These conflicting findings suggest that the response to dietary Ile is likely dependent on a multitude of environmental and nutritional factors, and further research should be conducted on the complex nature of Ile utilization and metabolism.

Bregendahl et al. (2008), Leeson and Summers (2005), and the CVB (1996) suggested that the ideal SID Ile:Lys ratio for laying hens is 79% (calculated from true digestible, total and digestible AA, respectively), which is lower than the 86% suggested by Coon and Zhang (1999) and the 94% (calculated from total AA) suggested by the NRC (1994). Rocha et al. (2013) found the optimal SID Ile:Lys ratio for laying hens aged 24 to 40 wk was between 82 and 84%. The same laboratory conducted a similar study in older hens aged 42 to 58 wk and concluded that the lowest ratio tested of 73% was sufficient for satisfactory performance, though performance was numerically improved as Ile level increased (Mello et al., 2012). The diets in the later study were not analyzed for CP content or AA profile; therefore, it is possible that Ile was not limiting. However, similar results are reported by Dong et al. (2016), where Lohmann Brown hens aged 28 to 40 wk showed no difference in performance when fed a 14.2% CP diet fortified with Ile between 72 and 123% total Ile to total Lys. Phase 2 results from the present study agree with these findings, in that the lowest level of dietary Ile was satisfactory to yield an EM and FCR similar to CON; however, optimal performance was observed when hens were provided an SID Ile:Lys ratio of 82%, similar to the findings of Rocha et al. (2013). These authors observed quadratic responses in both EM and FI to Ile supplementation suggesting that the lower FI associated with high dietary Ile compromised egg production. In the present study, feed consumption was not affected in phase 1; however, hens responded linearly in phase 2 with reduced FI in both Ile90 and Ile100 treatments. Hens fed Ile70 and Ile80 did not adjust FI compared to CON; therefore, energy intake was maintained, which

Table 6. Feed intake and production performance responses in White Shaver laying hens during phase 1 (20 to 27 wk) and 2 (28 to 46 wk) fed a low crude protein diet with varying levels of L-Ile compared to a control (**CON**).

Item	Feed intake (g/D)	Hen-day egg production (%)	Egg weight (g)	Egg mass (g)	Feed conversion ratio
Phase 1					
CON	97	97.6[a]	51.7[a,b]	50.6[a]	1.93
Ile70	95	94.3[b]	51.3[a,b]	48.6[b]	1.99
Ile80	98	93.8[b]	51.5[a,b]	48.6[b]	2.05
Ile90	96	95.4[a,b]	52.1[a]	49.9[a,b]	1.97
Ile100	96	97.6[a]	51.1[b]	49.8[a,b]	1.95
SEM	0.789	0.760	0.205	0.425	0.031
P-value	0.211	<0.001	0.004	0.003	0.097
Contrasts (*P*-value)					
CON vs. Ile70	0.124	0.003	0.114	<0.001	0.231
CON vs. Ile80	0.492	<0.001	0.443	0.001	0.011
Linear	0.493	0.005	0.782	0.035	0.387
Quadratic	0.126	0.039	0.002	0.960	0.183
Phase 2					
CON	114[a]	99.2[a,b]	58.2[a]	57.5	1.98[a,b]
Ile70	113[a,b]	97.7[c]	58.6[a]	57.2	1.98[a,b]
Ile80	116[a]	98.1[b,c]	58.0[a,b]	57.0	2.05[a]
Ile90	110[b]	99.6[a]	58.2[a]	58.0	1.90[c]
Ile100	111[b]	99.1[a,b]	57.3[b]	56.7	1.95[b,c]
SEM	0.967	0.302	0.210	0.325	0.020
P-value	<0.0001	<0.0001	0.001	0.058	<0.0001
Contrasts (*P*-value)					
CON vs. Ile70	0.739	<0.001	0.195	0.416	0.804
CON vs. Ile80	0.119	0.013	0.558	0.216	0.013
Linear	<0.001	<0.001	<0.001	0.695	0.002
Quadratic	0.043	0.003	0.138	0.006	0.001

[a–c]Values are statistically significant ($P < 0.05$).

Table 7. Nutrient intake of hens fed either a control (CON) or a low crude protein (LCP) diet fortified with graded levels of L-Ile.

Item	CON	Ile70	Ile80	Ile90	Ile100	SEM
Phase 1						
SID Ile intake (mg/D)	624[c]	570[d]	669[b]	675[b]	733[a]	4.43
SID Lys intake (mg/D)	737[c]	764[b]	793[a]	762[b]	739[c]	5.20
Ca intake (g/D)	3.70[b]	3.69[b]	4.09[a]	3.75[b]	3.48[c]	0.042
P intake (g/D)	0.61[b]	0.66[a]	0.66[a]	0.67[a]	0.61[b]	0.007
GE Intake (kcal/D)	349	335	357	341	346	2.37
Phase 2						
SID Ile intake (mg/D)	655[c]	584[e]	622[d]	691[b]	797[a]	5.44
SID Lys intake (mg/D)	891[a]	879[a]	868[a,b]	842[b,c]	837[c]	7.21
Ca intake (g/D)	3.96	4.39	5.08	4.38	4.12	0.052
P intake (g/D)	0.68	0.75	0.81	0.75	0.69	0.008
GE Intake (kcal/D)	411	389	391	376	376	3.35

Abbreviation: SID, standardized ileal digestible.
[a–c]Values are statistically significant ($P < 0.05$).

may explain why EW was sustained. At the end of the trial, FI of birds fed the LCP diets ranged from 104 to 119 g/D, with a calculated daily protein consumption of 17 g, which is similar to breeder recommendations. It should be noted, however, that spillage of feed was unavoidable, and although outliers were removed, the average FI could still be overestimated. Regardless, analyzed dietary Lys levels were higher than intended in phase 2 which may result in underestimated requirements for SID Ile:Lys due to excess Lys intake. Nutrient intake values were calculated from diet analysis and are shown in Table 7. In phase 1, LCP-fed hens that consumed from 675 to 733 mg SID Ile/D (88 to 99% SID Ile:Lys) yielded a slightly lower, but statistically similar EM to CON birds, who consumed an average of 624 mg SID Ile/D (85% SID Ile:Lys), with no differences in FCR. During phase 2, EM was maintained at the same level in all treatments; however, differences in FCR were observed with the best feed conversion in Ile90 birds, who had an average daily intake of 691 mg SID Ile (82% SID Ile:Lys).

Canadian and American eggs are priced according to egg size rather than EM output; however, there is no price advantage of producing eggs beyond 63 g due to concerns related to egg-shell integrity. Therefore, a more practical approach to assessing treatment effects on EW is to separate eggs according to size category, as producers aim to avoid producing extra-large and jumbo eggs to optimize income. Table 8 summarizes the proportion of eggs according to size for each phase, and egg quality and composition responses are shown in Tables 9 and 10, respectively. The most notable difference in egg size distribution is between Ile90 and Ile100, where the proportion of eggs shifted from primarily large-sized to mostly medium-sized eggs. Dietary treatment did not affect ($P > 0.05$) eggshell break strength; however, the shell component tended to decrease linearly ($P = 0.09$), confirming that egg size was getting smaller with increasing dietary Ile while maintaining similar eggshell integrity. A reduction of dietary CP resulted in eggs with a higher Y:A, primarily due to greater yolk size, and the addition of Ile to LCP diets linearly increased ($P < 0.001$) the albumen component (%) and height (mm) in the egg. Similarly, Haugh unit was improved with increasing dietary Ile. Some studies have shown similar results (Huyghebaert et al., 1991; Blair et al., 1999), whereas others reported no effect of changing CP or Ile levels on egg quality and composition (Casartelli et al., 2005; Rocha et al., 2013; Torki et al., 2015; Ospina Rojas et al., 2015). Limited information is available regarding the metabolic control of egg protein production; therefore, Ile's role in regulating egg size and composition is unknown and requires further investigation.

The intent of the experimental design was to have one LCP diet at the estimated requirement of 80% SID Ile:-Lys, one below, and 2 above to determine both the effect of protein reduction without additional Ile and to validate current recommendations. The CON treatment

Table 8. Distribution of eggs laid according to Canadian egg size category.

	Egg size[1] (% of total eggs laid)				
Diet	Peewee	Small	Medium	Large	XL & jumbo
Phase 1[2]					
CON	0.5	15.8	57.2	24.9[a,b]	1.6
Ile70	0.5	17.5	58.6	20.7[a,b]	2.7
Ile80	0.5	17.4	58.5	22.5[a,b]	1.1
Ile90	1.0	13.8	51.2	30.2[a]	3.7
Ile100	0.5	18.9	58.6	19.0[b]	3.0
SEM	0.248	2.02	3.31	2.14	1.01
P-value	0.431	0.471	0.462	0.013	0.396
Phase 2[2]					
CON	0.0	0.3	29.1[a,b]	57.3[a,b]	13.3[a]
Ile70	0.0	0.9	28.0[b]	57.8[a,b]	13.4[a]
Ile80	0.0	1.4	27.3[b]	63.8[a]	7.5[b]
Ile90	0.9	1.6	25.3[b]	63.0[a]	9.2[a,b]
Ile100	0.0	3.4	35.2[a]	54.0[b]	7.4[b]
SEM	0.41	0.86	1.75	2.24	1.39
P-value	0.419	0.138	0.004	0.018	0.004

Abbreviation: CON, control.
[a,b]Values are statistically significant ($P < 0.05$).
[1]Phase 1: 20 to 27 wk; phase 2: 28 to 46 wk of age.
[2]According to Egg Farmers of Canada: peewee, <42 g; small, 42≤>49; medium, 49≤>56; large, 56≤>63; XL, 63≤>70; jumbo, 70≥.

Table 9. Egg quality responses of hens during phase 1 (20 to 27 wk) and 2 (28 to 46 wk) as influenced by a 2%-unit CP reduction with varying levels of L-Ile.

Item	Breaking force (kgf)	Albumen height (mm)	Haugh unit
Phase 1			
CON	4.83	4.97	70.13
Ile70	4.87	5.10	72.14
Ile80	4.63	5.25	72.93
Ile90	4.86	5.19	72.46
Ile100	4.66	5.47	74.36
SEM	0.065	0.154	1.243
P-value	0.015	0.127	0.127
Contrasts (*P*-value)			
CON vs. Ile70	0.645	0.540	0.248
CON vs. Ile80	0.027	0.198	0.109
Linear	0.027	0.112	0.234
Quadratic	0.856	0.585	0.586
Phase 2			
CON	4.49	5.14[a,b]	68.33[a,b]
Ile70	4.54	4.20[b]	66.60[b]
Ile80	4.34	5.05[a,b]	67.59[a,b]
Ile90	4.56	5.28[a,b]	69.79[a,b]
Ile100	4.50	5.40[a]	71.59[a]
SEM	0.059	0.116	1.149
P-value	0.077	0.035	0.020
Contrasts (*P*-value)			
CON vs. Ile70	0.540	0.172	0.281
CON vs. Ile80	0.079	0.594	0.649
Linear	0.290	0.002	0.001
Quadratic	0.383	0.536	0.781

Abbreviations: CON, control; CP, crude protein.
[a,b]Values are statistically significant ($P < 0.05$).

was also formulated to meet a SID Ile:Lys of 80% to determine whether crystalline AA can effectively replace intact protein from raw ingredients. In phase 1, Ile80 had similar proportions of Lys, Met, Thr, Trp, Val, and Ile as CON, yet HDEP and EM was lower, thus going against the suggestion that performance can be maintained with CP reduction as long as the ideal AA profile is being met (Ji et al., 2014). Although the SID Ile:Lys was lower in phase 2, CON birds maintained optimal performance, whereas Ile80 birds continued to perform poorly. Digestibility coefficients for AA were obtained from broiler chick assays; therefore, it is possible that the SID AA levels of the treatments were incorrectly estimated. Studies that compared ileal digestibilities of different ingredients between layers and broilers indicated a diverse digestive capacity, depending on the composition of the diet; however, not enough data are available to construct a separate table for laying hens (Huang et al., 2006; Adedokun et al., 2009, 2014, 2015). Variations in AA level and digestibility also exist between different ingredient sources, and inconsistencies have been reported in standardized AA digestibility values among different assay methods (Kim et al., 2011, 2012a,b). Average daily consumption of Ile was also different between Ile80 and CON in both phases, and Lys consumption was in excess during phase 2, making it difficult to verify the ideal ratio or absolute daily requirements. It is also possible that the variation in dietary calcium levels may have confounded diet effects. Calcium contributes to buffering capacity in the gizzard; therefore, an increased pH may have compromised protein digestion which may partially explain the reduced performance of Ile80 (Mutucumarana et al., 2014). To develop a reliable feed ingredient database for poultry, more information is needed on how nutrient digestibility is influenced by physiological differences and nutrient interactions.

Our current understanding of AA requirements of the modern laying hen remains limited, and reports on the effects of LCP diets fortified with AA on layer hen performance are inconsistent. Furthermore, the metabolic effects and interactions that occur between the different AA, such as the branched chain AA (**BCAA**; Ile, Leu, Val), are not well understood. The primary objective of this trial was to evaluate production performance and egg quality responses to graded levels of synthetic Ile in layer diets reduced by 2% units in CP from the commercial standard. Under the conditions that all other AA requirements are met, the results indicate that a corn-SBM based diet with CP reduced by 2% unit points is limiting in Ile and will compromise HDEP, whereas excess L-Ile will limit EW, possibly indicating a shift in the utilization of dietary AA.

Table 10. Egg composition of hens fed a low crude protein (LCP) diet fortified with varying levels of L-Ile compared to a high crude protein (HCP) control during phase 2 (28 to 46 wk of age).

	Absolute weight (g)			Proportion (%)			
Item	Albumen	Yolk	Shell	Albumen	Yolk	Shell	Y:A[1]
CON	36.63	14.84[b]	6.81[a]	62.68[a,b]	25.45[b]	11.87[a]	0.407[b]
Ile70	36.29	15.44[a]	6.66[a,b]	61.92[b]	26.36[a]	11.72[a,b]	0.427[a]
Ile80	36.21	15.38[a]	6.53[b]	62.23[a,b]	26.39[a]	11.38[b]	0.425[a]
Ile90	36.37	15.07[a,b]	6.57[a,b]	62.69[a,b]	25.95[a,b]	11.36[b]	0.415[a,b]
Ile100	35.83	14.72[b]	6.44[b]	62.86[a]	25.81[a,b]	11.32[b]	0.411[a,b]
SEM	0.296	0.141	0.063	0.199	0.185	0.121	0.004
P-value	0.425	<0.001	<0.001	0.005	0.001	0.002	0.002
Contrasts (*P*-value)							
CON vs. Ile70	0.407	0.003	0.088	0.007	<0.001	0.358	<0.001
CON vs. Ile80	0.309	0.008	0.002	0.113	<0.001	0.004	0.002
Linear	0.338	<0.001	0.048	<0.001	0.009	0.085	0.002
Quadratic	0.341	0.867	0.540	0.358	0.622	0.448	0.488

Abbreviation: CON, control.
[a,b]Values are statistically significant ($P < 0.05$).
[1]Yolk: Albumin.

Furthermore, broiler digestibility values of feedstuffs likely do not apply to the modern laying hen, and the utilization of dietary AA may vary between intact protein and free crystalline AA. The potential economic benefit for producers feeding LCP diets largely depends on current price of eggs, value difference between medium and large eggs, and cost of synthetic AA/feed ingredients. Whether the differences in egg production performance is a function of an adjusted Ile:Lys ratio, altering the BCAA ratio, or the bioavailability of crystalline vs. intact AA, remains unknown and should be further investigated.

ACKNOWLEDGMENTS

Supported by Ontario Agri-Food Innovation Alliance, Ontario Centers of Excellence, Natural Sciences and Engineering Research Council of Canada (Engage program), and Halchemix. Technical assistance by Ajinomoto Animal Nutrition Group is appreciated. Ilona A. Parenteau was a recipient of the Ontario Graduate Student Scholarship.

REFERENCES

Adedokun, S., P. Utterback, C. Parsons, O. Adeola, M. Lilburn, and T. Applegate. 2009. Comparison of amino acid digestibility of feed ingredients in broilers, laying hens and caecectomised roosters. Br. Poult. Sci. 50:350–358.

Adedokun, S., P. Jaynes, M. Adb El-Hack, R. Payne, and T. Applegate. 2014. Standardized ileal amino acid digestibility of meat and bone meal and soybean meal in laying hens and broilers. Poult. Sci. 93:420–428.

Adedokun, S., P. Jaynes, R. Payne, and T. Applegate. 2015. Standardized ileal amino acid digestibility of corn, corn distillers' dried grains with solubles, wheat middlings, and bakery by-products in broilers and laying hens. Poult. Sci. 94:2480–2487.

Bain, M. M., Y. Nys, and I. C. Dunn. 2016. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? Br. Poult. Sci. 57:330–338.

Baker, D., A. Batal, T. Parr, N. Augspurger, and C. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81:185–494.

Berres, J., S. L. Vieira, M. T. Kidd, D. Taschetto, D. M. Freitas, R. Barros, and E. T. Nogueira. 2010. Supplementing l-valine and l-isoleucine in low-protein corn and soybean meal all-vegetable diets for broilers. J. Appl. Poult. Res. 19:373–379.

Blair, R., J. Jacob, S. Ibrahim, and P. Wang. 1999. A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. J. Appl. Poult. Res. 8:25–47.

Bray, D. J. 1964. Studies with corn-soya laying diets: 7. Limiting amino acids in a 60:40 blend of corn and soybean protein. Poult. Sci. 43:396–401.

Bray, D. J. 1969. Studies with corn-soya laying diets 8. Requirements for limiting amino acids—the basal diet and the requirements for isoleucine, lysine and tryptophan. Poult. Sci. 48:674–684.

Bregendahl, K., S. Roberts, B. Kerr, and D. Hoehler. 2008. Ideal ratios of isoleucine, methionine, methionine plus cystine, threonine, tryptophan, and valine relative to lysine for white leghorn-type laying hens of twenty-eight to thirty-four weeks of age. Poult. Sci. 87:744–758.

Burley, H., P. Patterson, and M. Elliot. 2013. Effect of a reduced crude protein, amino acid-balanced diet on hen performance, production costs, and ammonia emissions in a commercial laying hen flock. J. Appl. Poult. Res. 22:217–228.

Canadian Council on Animal Care (CCAC). 2009. CCAC guidelines on: the care and use of farm animals in research, teaching and testing. Accessed Mar. 2018. Ottawa ON, Canada. https://www.ccac.ca/Documents/Standards/Guidelines/Farm_Animals.pdf.

Casartelli, E., R. Filardi, O. Junqueira, A. Laurentiz, V. Assuena, and K. Duarte. 2005. Commercial laying hen diets formulated according to different recommendations of total and digestible amino acids. Braz. J. Poult. Sci. 7:177–180.

Centraal Veevoederbureau (CVB). 1996. Aminozurenbohoefte van leghennen en vleeskuikens [Amino Acid Requirements for Laying Hens and Broiler Chickens]. Centraal Veevoederbureau, Lelystad, the Netherlands.

Coon, C., and B. Zhang. 1999. Ideal amino acid profile for layers examined. Feedstuffs 71:13–15.

Corzo, A., R.E. Loar, II, and M. T. Kidd. 2009. Limitations of dietary isoleucine and valine in broiler chick diets1. Poult. Sci. 88:1934–1938.

Dong, X. Y., M. M. M. Azzam, and X. T. Zou. 2016. Effects of dietary L-isoleucine on laying performance and immunomodulation of laying hens. Poult. Sci. 95:2297–2305.

Egg Farmers of Canada. 2017. Egg Farmers of Canada annual report. Accessed Jan. 2019. https://www.eggfarmers.ca/wp-content/uploads/2018/03/2017_Egg-Farmers-of-Canada-Annual-Report.pdf.

Egg Farmers of Canada. 2018. Egg Farmers of Canada annual report. Accessed June 2019. https://www.eggfarmers.ca/wp-content/uploads/2019/03/2018_Egg-Farmers-of-Canada-Annual-Report.pdf.

Elliot, M. 2008. Amino acid nutrition of commercial pullets and layers. Pages 193–221 in Proceedings of Intermountain Nutrition Conference. Utah Agricultural Experiment Station, Salt Lake City, UT, pp. 193–221.

Harms, R., and F. Ivey. 1993. Performance of commercial laying hens fed various supplemental amino acids in a corn-soybean meal diet. J. Appl. Poult. Res. 2:273–282.

Harms, R., and G. Russel. 1993. Optimizing egg mass with amino acid supplementation of a low-protein diet. Poult. Sci. 72:1892–1896.

Harms, R. H., and G. B. Russell. 2000. Evaluation of the isoleucine requirement of the commercial layer in a corn-soybean meal diet. Poult. Sci. 79:1154–1157.

Hendrix ISA. 2011. Nutrition Management Guide. 2011 ed. ISA Hendrix Genetics, Saint Brieuc, France.

Huang, K., X. Li, V. Ravindran, and W. Bryden. 2006. Comparison of apparent ileal amino acid digestibility of feed ingredients measured with broilers, layers and roosters. Poult. Sci. 85:625–634.

Huyghebaert, G., G. De Groote, E. A. Butler, and T. R. Morris. 1991. Optimum isoleucine requirement of laying hens and the effect of age. Br. Poult. Sci. 32:471–481.

Jensen, L. S. 1991. Protein and amino acid nutrition of laying hens and broilers. Pages 159–170 in Proceedings of the 26th Annual Northwest Animal Nutrition Conference. Henschel, K.K., ed. Beaverton, OR.

Ji, F., S. Fu, B. Ren, S. Wu, H. Zhang, H. Yue, J. Gao, A. Helmbrecht, and G. Qi. 2014. Evaluation of amino-acid supplemented diets varying in protein levels for laying hens. J. Appl. Poult. Res. 23:384–392.

Keshavarz, K., and M. Jackson. 1992. Performance of growing pullets and laying hens fed low-protein, amino acid-supplemented diets. Poult. Sci. 71:905–918.

Kim, E. J., P. L. Utterback, T. J. Applegate, and C. M. Parsons. 2011. Comparison of amino acid digestibility of feedstuffs determined with the precision-fed cecectomized rooster assay and the standardized ileal amino acid digestibility assay. Poult. Sci. 90:2511–2519.

Kim, E. J., P. L. Utterback, and C. M. Parsons. 2012a. Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among 3 different bioassays. Poult. Sci. 91:1350–1355.

Kim, E. J., P. L. Utterback, and C. M. Parsons. 2012b. Comparison of amino acid digestibility coefficients for corn, corn gluten meal, and corn distillers dried grains with solubles among 3 different bioassays. Poult. Sci. 91:3141–3147.

Kristensen, H., and C. Wathes. 2000. Ammonia and poultry welfare: a review. Worlds Poult. Sci. 56:235–245.

Leeson, S., and J. D. Summers. 2005. Commercial Poultry Production, 3rd ed. University Books, Guelph, ON.

de Lima, M., N. Sakomura, J. Dorigam, E. da Silva, N. Ferreira, and J. Fernandes. 2016. Maintenance valine, isoleucine and tryptophan requirements for poultry. Poult. Sci. 95:842–850.

de Lima, M., N. K. Sakomura, E. Silva, J. C. P. Dorigam, N. T. Ferreira, E. Malheiros, and J. Fernandes. 2018. The optimal digestible valine, isoleucine and tryptophan intakes of broiler breeder hens for rate of lay. Anim. Feed Sci. Technol. 238:29–38.

Mello, H. H. de C., P. C. Gomes, T. C. da Rocha, J. L. Donzele, R. L. de Almeida, A. R. Troni, B. R. de Carvalho, and G. da S. Viana. 2012. Determination of digestible isoleucine: lysine ratio in diets for laying hens aged 42-58 weeks. Rev. Bras. Zootec. 41:1313–1317.

Meluzzi, A., F. Sirri, N. Tallarico, and A. Franchini. 2001. Nitrogen retention and performance of brown laying hens on diets with different protein content and constant concentration of amino acids and energy. Br. Poult. Sci. 42:213–217.

Miller, E., M. Sunde, H. Bird, and C. Elvehjem. 1954. The isoleucine requirements of the laying hen. Poult. Sci. 33:1201–1209.

Morris, T., and R. Gous. 1988. Partitioning of the response to protein between egg number and egg weight. Br. Poult. Sci. 29:93–99.

Mutucumarana, R., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Influence of dietary calcium concentration on the digestion of nutrients along the intestinal tract of broiler chickens. J. Poult. Sci. 51:392–401.

Mwaniki, Z., M. Neijat, and E. Kiarie. 2018. Egg production and quality responses of adding up to 7.5% defatted black soldier fly larvae meal in a corn–soybean meal diet fed to Shaver White Leghorns from wk 19 to 27 of age. Poult. Sci. 97:2829–2835.

National Research Council. 1994. Nutrient Requirements of Poultry, 9th rev. ed. National Academy Press, Washington, DC.

Ospina Rojas, I., A. Murakami, J. Fanhani, K. Picoli, and M. Baptista Barbosa. 2015. Tryptophan, threonine and isoleucine supplementation in low-protein diets for commercial laying hens. Semin. Ciênc. Agrár. 36:1735–1744.

Peganova, S., F. Hirche, and K. Eder. 2003. Requirement of tryptophan in relation to the supply of large neutral amino acids in laying hens. Poult. Sci. 82:815–822.

Roberts, S., H. Xin, B. Kerr, J. Russell, and K. Bregendahl. 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. Poult. Sci. 86:1716–1725.

Rocha, T. C. da, J. L. Donzele, P. C. Gomes, H. H. de C. Mello, A. R. Troni, and G. da S. Viana. 2013. Ideal digestible isoleucine: digestible lysine ratio in diets for laying hens aged 24-40 weeks. Rev. Bras. Zootec. 42:780–784.

Shivazad, M., R. Harms, G. Russell, D. Faria, and R. Antar. 2002. Re-evaluation of the isoleucine requirement of the commercial layer. Poult. Sci. 81:1869–1872.

Sohail, S., M. Bryant, and D. Roland. 2002. Influence of supplemental lysine, isoleucine, threonine, tryptophan and total sulfur amino acids on egg weight of Hyline W-36 hens. Poult. Sci. 81:1038–1044.

Summers, J. D. 1993. Reducing nitrogen excretion of the laying hen by feeding lower crude protein diets. Poult. Sci. 72:1473–1478.

Torki, M., M. Ahmad, H. A. Ghasemi, and A. Zardast. 2015. Response of laying hens to feeding low-protein amino acid-supplemented diets under high ambient temperature: performance, egg quality, leukocyte profile, blood lipids and excreta pH. Int. J. Biometeorol. 59:575–584.

USDA. 2019. World agricultural supply and demand estimates. https://www.usda.gov/oce/commodity/wasde/wasde1019.pdf.