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

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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 g/C20 EW 63 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)1 Ile, with optimal responses observed between 82 and 88% SID Ile:Lys.

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

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

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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 Russell, 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 kg ± 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...
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 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-68%         | 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 premix2 | 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   |
| Gln                   | 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 digestsible.

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 mcg; thiamin, 400 mcg; riboflavin, 800 mcg; pantothenic acid, 2,000 mcg; pyridoxine, 430 mcg; niacin, 65,000 mcg; folic acid, 220 mcg; choline, 60,000 mcg; iron, 6,000 mcg; copper, 1,000 mcg; manganese, 7,000 mcg; zinc, 7,000 mcg, iodine, 100 mcg.
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 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).

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

| 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  | 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                      | 2.0   | 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  | 0.75  | 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                       | 0.06  | 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.

1CON: 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.

2Vitamins 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, 500 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.

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
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 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.

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.
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

| Diet  | Calculated | Analyzed total | Analyzed SID\(^1\) |
|-------|------------|----------------|-------------------|
|       | Total | SID\(^2\) | Phase 1\(^3\) | Phase 2\(^4\) | 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.

| 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\(^b\)     | 50.6\(^c\)  | 1.93                 |
| Ile70              | 95               | 94.3\(^b\)                | 51.3\(^b\)     | 48.6\(^c\)  | 1.99                 |
| Ile80              | 98               | 93.8\(^b\)                | 51.5\(^b\)     | 48.6\(^c\)  | 2.05                 |
| Ile90              | 96               | 95.4\(^b\)                | 52.1\(^c\)     | 49.9\(^b\)  | 1.97                 |
| Ile100             | 96               | 97.6\(^c\)                | 51.1\(^c\)     | 49.8\(^b\)  | 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\(^d\)        | 99.2\(^b\)                | 58.2\(^a\)     | 57.5         | 1.98\(^b\)           |
| Ile70              | 113\(^b\)        | 97.7\(^e\)                | 58.6\(^c\)     | 57.2         | 1.98\(^b\)           |
| Ile80              | 110\(^c\)        | 98.1\(^c\)                | 58.0\(^b\)     | 57.0         | 2.05\(^a\)           |
| Ile90              | 110\(^d\)        | 99.6\(^c\)                | 58.2\(^a\)     | 58.0         | 1.90\(^c\)           |
| Ile100             | 111\(^e\)        | 99.1\(^b\)                | 57.3\(^c\)     | 56.7         | 1.95\(^d\)           |
| 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 \((P < 0.05)\).
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 effects on EW is to separate eggs according to size category. 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 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 | 570   | 669   | 675   | 733    | 4.43 |
| SID Lys intake (mg/D)       | 737 | 764   | 793   | 762   | 739    | 5.20 |
| Ca intake (g/D)             | 3.70| 3.69  | 4.09  | 3.75  | 3.48   | 0.042|
| P intake (g/D)              | 0.61| 0.66  | 0.66  | 0.67  | 0.61   | 0.007|
| GE intake (kcal/D)          | 349 | 335   | 357   | 341   | 346    | 2.37 |
| Phase 2                     |     |       |       |       |        |      |
| SID Ile intake (mg/D)       | 655 | 584   | 622   | 691   | 797    | 5.44 |
| SID Lys intake (mg/D)       | 891 | 879   | 808   | 812   | 837    | 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.

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

| Diet   | Peewee | Small | Medium | Large | XL & jumbo |
|--------|--------|-------|--------|-------|------------|
| Phase 1|        |       |        |       |            |
| CON    | 0.5    | 15.8  | 57.2   | 24.9  | 1.6        |
| Ile70  | 0.5    | 17.5  | 58.6   | 20.7  | 2.7        |
| Ile80  | 0.5    | 17.4  | 58.5   | 22.5  | 1.1        |
| Ile90  | 1.0    | 13.8  | 51.2   | 30.2  | 3.7        |
| Ile100 | 0.5    | 18.9  | 58.6   | 19.0  | 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|        |       |        |       |            |
| CON    | 0.0    | 0.3   | 29.1   | 57.3  | 13.3       |
| Ile70  | 0.0    | 0.9   | 28.0   | 57.8  | 13.4       |
| Ile80  | 0.0    | 1.4   | 27.4   | 63.8  | 7.5        |
| Ile90  | 0.9    | 1.6   | 25.3   | 63.0  | 9.2        |
| Ile100 | 0.0    | 3.4   | 35.2   | 54.0  | 7.4        |
| 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.

1Values are statistically significant (P < 0.05).

2According to Egg Farmers of Canada: peewee, <42 g; small, 42≤>49; medium, 49≤>56; large, 56≤>63; XL, 63≤>70; jumbo, 70≥.
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 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<sup>ab</sup>   | 68.33<sup>ab</sup> |
| Ile70         | 4.54                | 4.29<sup>b</sup>    | 66.60<sup>b</sup> |
| Ile80         | 4.34                | 5.05<sup>ab</sup>   | 67.59<sup>b</sup> |
| Ile90         | 4.56                | 5.26<sup>ab</sup>   | 69.79<sup>b</sup> |
| Ile100        | 4.50                | 5.40<sup>a</sup>    | 71.59<sup>a</sup> |
| 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.
<sup>a,b</sup>Values are statistically significant (<i>P</i> < 0.05).

### 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).

| Item          | Absolute weight (g) | Proportion (%) |
|---------------|---------------------|----------------|
|               | Albumen | Yolk | Shell | Albumen | Yolk | Shell | Y:A<sup>1</sup> |
| CON           | 36.63   | 14.84<sup>b</sup> | 6.81<sup>a</sup> | 62.68<sup>b</sup> | 25.45<sup>b</sup> | 11.87<sup>a</sup> | 0.407<sup>b</sup> |
| Ile70         | 36.29   | 15.44<sup>a</sup> | 6.66<sup>b</sup> | 61.92<sup>b</sup> | 26.30<sup>a</sup> | 11.72<sup>b</sup> | 0.427<sup>a</sup> |
| Ile80         | 36.21   | 15.38<sup>a</sup> | 6.53<sup>b</sup> | 62.23<sup>b</sup> | 26.39<sup>a</sup> | 11.38<sup>b</sup> | 0.425<sup>a</sup> |
| Ile90         | 36.37   | 15.07<sup>b</sup> | 6.57<sup>a</sup> | 62.69<sup>b</sup> | 25.95<sup>a</sup> | 11.36<sup>b</sup> | 0.415<sup>ab</sup> |
| Ile100        | 35.83   | 14.72<sup>b</sup> | 6.44<sup>a</sup> | 62.86<sup>a</sup> | 25.81<sup>a</sup> | 11.32<sup>b</sup> | 0.411<sup>b</sup> |
| 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.
<sup>a,b</sup>Values are statistically significant (<i>P</i> < 0.05).
<sup>1</sup>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.

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