

Evaluation of the Isoleucine Requirement of the Commercial Layer in a Corn-Soybean Meal Diet¹

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ABSTRACT An experiment was conducted with Hy-Line W36 hens to evaluate their Ile requirement in a corn-soybean meal diet. Five experimental diets were fed with Ile levels of 0.61, 0.58, 0.55, 0.52, and 0.49%. Supplemental amino acids (AA) were added to ensure that Ile was the first-limiting AA. Two diets with 0.55 and 0.52% Ile were also fed with higher levels of supplemental AA. A positive control (0.67% Ile) with only Met supplementation was

fed, which had previously been shown to support maximum performance. All levels of Ile addition significantly increased egg production (EP), egg weight (EW), and egg contents (EC).

Broken-line regression indicated a daily Ile requirement of 589.2, 601.2, and 601.4 mg per day for EP, EW, and EC, respectively, which indicated a requirement of 12.6 mg Ile per gram of EC.

(*Key words:* commercial layer, isoleucine, egg production, egg content)

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INTRODUCTION

Isoleucine is one of the limiting amino acids (AA) in a low-protein, corn-soybean layer diet. In 1971, the National Research Council (NRC, 1971) suggested the diet of a commercial layer should contain 0.50% Ile. Later, the suggested requirement was changed to 550 mg per hen per day (NRC 1977, 1984), and the last suggestion (NRC 1994) was 650 mg per day.

There are limited data for making these suggestions. Miller et al. (1954) suggested that approximately 0.53% L-isoleucine was required in the diet of the laying hen to support egg production (EP) and BW when blood meal was the main source of protein. Maximum EP was 80% at the start of their experiment and it decreased to 60% with the control hens after 2 wk.

Bray (1969) used a basal diet consisting of 8.5% protein with 60% of the protein from corn and 40% from soybean meal. He reported that least squares analysis of the data indicated a dietary Ile requirement of 0.397% and a daily Ile requirement of 472 mg per hen. The hens in that experiment laid at approximately 80% with a daily egg mass of 45 g.

Gous et al. (1987) conducted an experiment using the diet dilution technique to evaluate the relationship of dietary energy and Ile concentrations. They regressed mean egg output on mean intake of Ile. It appeared that

the daily Ile requirement was between 650 and 700 mg per hen per day.

Harms and Ivey (1993) reported no response from the addition of Ile to a corn-soybean meal diet containing supplemental Met, Lys, Trp, Arg, and Thr. The hens receiving this diet had a daily intake of 469 mg of Ile. Without the supplemental Ile they laid at 82.5% with 55.1 g egg weight (EW), which resulted in an egg content (EC) of 40.91 g, and an Ile intake of 11.46 mg per gram of EC (469/40.91).

Coon and Zhang (1999) estimated that a hen's daily requirement for digestible Ile is 579 mg. Schutte (1998) recommended a daily intake of 550 mg of digestible Ile or an intake of 660 mg of total Ile per hen per day.

It is common practice to use supplemental AA in layer feeds. A large percentage of the layer feed is based on corn and soybean meal. Methionine is the first-limiting AA in these diets, and Lys is the second (Harms and Ivey, 1993). Synthetic forms of these AA are available and that of Met is routinely used. A limited amount supplemental Lys is used. Tryptophan is the third-limiting AA in a corn-soybean meal diet (Russell and Harms, 1999) and is commercially available. However, little supplemental Trp is used, primarily because information is not available on the fourth-limiting AA. The fourth-limiting AA will be Ile, Val, or Thr (Harms and Ivey, 1993). Information is needed for the requirements of these AA to determine the amount of supplemental Trp that can be used before the fourth AA becomes limiting. Therefore, the present

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Abbreviation Key: AA = amino acids; EC = egg content; EP = egg production; EW = egg weight.

TABLE 1. Composition of diets

Ingredient	Diet							
	1	2	3	4	5	6	7	8
	0.67	0.61	0.58	0.55	0.52	0.49	0.55 ⁵	0.52 ⁶
	(%)							
Yellow corn	64.049	69.663	70.654	72.191	73.733	75.270	72.038	73.575
Soybean meal (48%)	25.148	20.324	19.239	17.694	16.148	14.603	17.710	16.165
Limestone	7.673	7.652	7.647	7.641	7.634	7.628	7.640	7.633
Dicalcium Phosphorus ¹	1.146	1.237	1.259	1.287	1.316	1.344	1.289	1.318
Vitamin mix ²	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Mineral mix ³	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Salt	0.378	0.378	0.378	0.378	0.378	0.378	0.378	0.378
DL-Met	0.106	0.108	0.113	0.102	0.096	0.083	0.120	0.109
Lys-HCl	...	0.097	0.127	0.128	0.128	0.129	0.178	0.179
Thr	...	0.013	0.030	0.026	0.123	0.020	0.054	0.050
Val	...	0.025	0.046	0.046	0.036	0.037	0.076	0.077
Trp	...	0.009	0.007	0.006	0.006	0.009	0.017	0.016
Calculated analysis ⁴								
Protein	16.42	14.66	14.29	13.69	13.07	12.47	13.79	13.18
Met	0.360	0.340	0.340	0.323	0.310	0.290	0.340	0.323
Lys	0.875	0.820	0.820	0.780	0.740	0.700	0.820	0.780
Trp	0.210	0.180	0.180	0.170	0.160	0.153	0.180	0.170
Thr	0.594	0.534	0.534	0.507	0.481	0.454	0.534	0.507
Val	0.770	0.700	0.700	0.670	0.630	0.600	0.700	0.670
Ile	0.670	0.610	0.580	0.550	0.520	0.490	0.550	0.520
Energy (kcal ME/kg)	2,797	2,839	2,849	2,862	2,875	2,889	2,863	2,876

¹Contains 18.5% P and 21% Ca.

²Supplies per kilogram of diet: biotin, 0.2 mg; cholecalciferol, 2,200 IU; choline, 500 mg; ethoxyquin, 65 mg; folic acid, 1 mg; niacin, 60 mg; pantothenic acid, 15 mg; pyridoxine, 5 mg; riboflavin, 5 mg; thiamin, 3 mg; vitamin A, 8,000 IU; vitamin B₁₂, 0.02 mg; vitamin E, 20 IU; and vitamin K, 2 mg.

³Supplies per kilogram of diet: copper, 10 mg; ethoxyquin, 65 mg; iodine, 2 mg; iron, 60 mg; manganese, 90 mg; selenium, 0.2 mg; and zinc, 80 mg.

⁴Based on analysis of corn and soybean meal.

⁵Same Ile as Diet 4 with higher levels of other amino acids.

⁶Same Ile as Diet 5 with higher levels of other amino acids.

experiment was conducted to evaluate the requirement for Ile in a corn-soybean meal diet.

MATERIALS AND METHODS

Hy-Line W36³ hens, 36 wk of age, were used in this experiment. They were housed one bird per cage (25.6 × 46.2 cm) in a windowless, fan-ventilated house. The temperature of the house was not allowed to fall below 26.7 C. Therefore, the temperature was almost constant because the experiment was conducted in February and March. The temperature was controlled to get a uniform feed intake at a level that an Ile deficiency could be produced. The photoperiod was 16 h/d.

Eight experimental diets were used (Table 1). A positive control diet (Diet 1) containing 0.67% Ile was used, which we have previously found to support maximum performance, and it contained supplemental Met. Diet 2 contained 0.61% Ile and other supplemental AA, which previously had been shown to support maximum performance (Harms and Russell, 1996a). Diet 3 contained 0.58% Ile (95.1% of Diet 2), and all other AA were maintained at 100% of Diet 2. Diet 4 contained 0.55% Ile (90.2% of

Diet 2), and the other AA were adjusted to 95% of Diet 2. Diet 5 contained 0.52% Ile (85% of Diet 2), and the levels of AA were held at 90% of Diet 2. Diet 6 contained 0.49% Ile (80.3% of Diet 2), and all other AA was held at 85% of Diet 2. Diets 7 and 8 were fed to test the ability of the level the other AA to support the performance of Diets 4 and 5, respectively. Diet 7 contained 0.55% Ile (90.2% of Diet 2 and 100% of the other AA in Diet 2). Diet 8 contained 0.52% Ile (85% of the Ile in Diet 2 and 95% of the other AA). These diets ensured that Ile was first-limiting in each diet. The procedure of reducing other AA when Ile was reduced was to eliminate a large excess of the other AA, but the diets would still contain an adequate amount of other AA. Eight replicates of five hens each were fed each diet. The Ile in all diets were furnished by corn and soybean meal. Amino acid analyses were determined for corn and soybean meal and were the basis for calculated values of diets.

Egg production was recorded for individual hens but was analyzed on a replicate basis. Feed consumption was measured biweekly, and remaining feed was replaced with fresh feed at that time. One egg from each hen, laid on the last 2 d of each week, was weighed. Eggs were broken out; the shells were washed, allowed to air dry, and were weighed. Egg mass was calculated by multiplying percentage EP by EW for each replicate. Egg con-

³Hy-Line International, West Des Moines, IA 50265.

TABLE 2. Performance of commercial layers fed various levels of Ile from 36 to 44 wk of age¹

Diet	Dietary Ile	Egg production	Egg weight	Egg content	Feed consumption	BW change	Ile intake	
							(h/d)	(per g EC)
	(%)	(%)	(g)	(g)	(g/h/d)	(g)	(mg)	(mg)
1	0.67	88.6 ^{ab}	59.3 ^a	47.8 ^a	96.5 ^a	91 ^a	671 ^a	14.1 ^a
2	0.61	86.9 ^{abc}	59.8 ^a	47.3 ^a	97.2 ^a	99 ^a	593 ^b	12.5 ^b
3	0.58	89.4 ^a	58.6 ^a	47.8 ^a	96.3 ^a	82 ^a	558 ^c	11.7 ^c
4	0.55	86.9 ^{abc}	58.3 ^a	46.1 ^{ab}	95.0 ^a	91 ^a	522 ^{de}	11.3 ^c
5	0.52	87.7 ^{abc}	58.4 ^a	46.9 ^{ab}	95.8 ^a	54 ^{ab}	498 ^{ef}	10.6 ^{de}
6	0.49	84.8 ^c	58.2 ^a	45.0 ^b	93.5 ^a	22 ^b	458 ^g	10.2 ^e
7	0.55 ²	87.1 ^{abc}	58.9 ^a	47.0 ^{ab}	97.7 ^a	100 ^a	537 ^{cd}	11.5 ^c
8	0.52 ³	86.2 ^{bc}	59.3 ^a	46.1 ^{ab}	94.4 ^a	77 ^a	491 ^f	10.8 ^d

^{a-g}Means with the same superscript within a column do not differ significantly ($P < 0.05$).

¹The diets were fed for 8 wk; however, only the last 6 wk were used to measure treatment effect.

²Contains 10% more of amino acids than the diet with 0.55% Ile.

³Contains 10% more of amino acids than the diet with 0.52% Ile.

tent was calculated by multiplying EP by EW minus shell weight. The hens were individually weighed at the beginning and end of the experiment, and weight change was calculated. The daily intake of Ile and energy were calculated by multiplying the concentration in the feed-by-feed consumption. Kilocalories of energy and milligrams of Ile per gram of EC were calculated by dividing the daily intake by daily EC. The experiment was conducted for 8 wk; however, the first 2 wk were considered as a depletion period, and Weeks 3 to 8 were used to test experimental treatments. This procedure has been used in our laboratory for AA studies (Harms and Russell, 1996b).

The data were subjected to ANOVA with the general linear model procedure of SAS (1990). Duncan's multiple-range test (1955) was used to determine significant differences among treatment means. The Ile requirements for EP, EC, and BW change were determined by using broken-line regression as described by Noll and Waibel (1989) for Treatments 2 through 8.

RESULTS AND DISCUSSION

Egg production increased from 84.8% when the hens received the diet with 0.49% Ile to 89.4% when the diet contained 0.58% Ile (Table 2). Mean EP from the hens receiving diets with 0.58 and 0.61% Ile was not different from the EP from hens receiving the practical diet with 0.67% Ile. Broken-line regression of EP on the Ile intake resulted in a requirement of 589.2 ± 48.4 ($R^2 = 0.15$) mg/h per d (Figure 1).

Egg weights increased from 58.2 g when the diet contained 0.49% Ile to 59.8 g when the diet contained 0.61% Ile (Table 2). This difference was not significant due to the large variation in EW. Broken-line regression of EW on Ile intake resulted in a requirement of 601.2 ± 45 mg/h per d ($R^2 = 0.20$) (Figure 2).

Egg content followed the same trend as EP and EW (Table 2). However, the EC from hens receiving the diet with 0.49% Ile was significantly less than the EC from hens receiving the diets with 0.58, 0.61, or 0.67% Ile. Broken-line

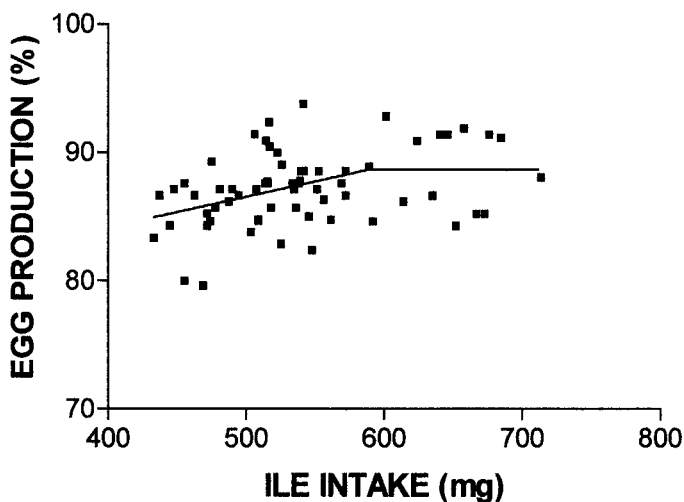


FIGURE 1. Broken-line regression of Ile on egg production $y = 88.69 + 0.0242 (X - 589.2)$.

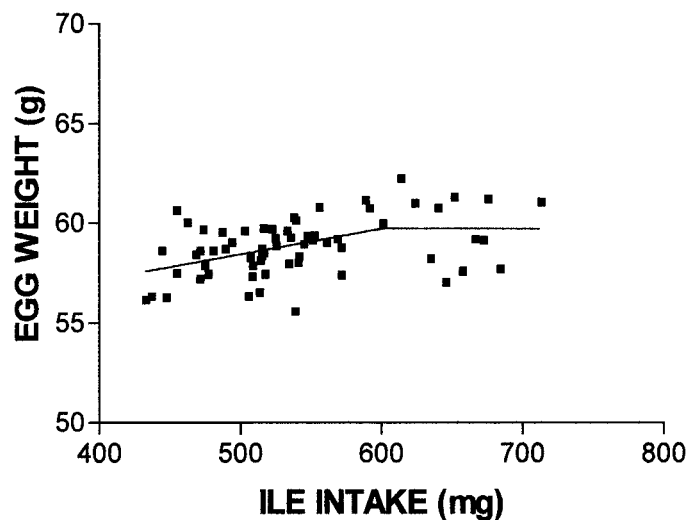


FIGURE 2. Broken-line regression of Ile on egg weight $y = 59.76 + 0.01291 (X - 601.2)$.

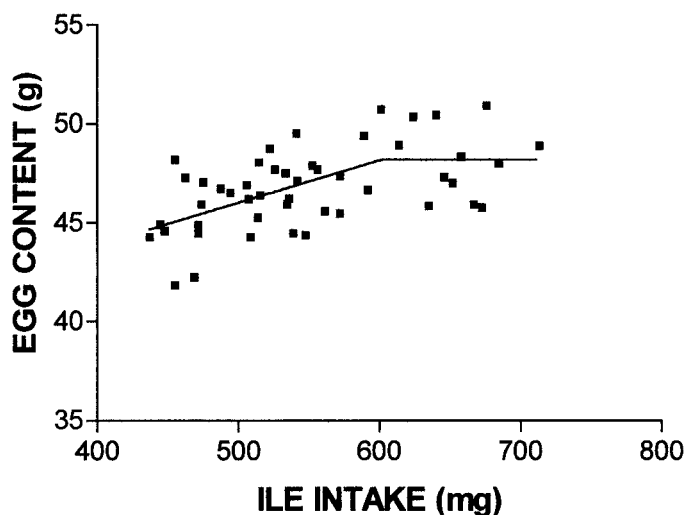


FIGURE 3. Broken-line regression of Ile on egg content $y = 48.27 + 0.0247(X - 601.4)$.

regression of EW on Ile intake indicated a requirement of 601.4 ± 33 mg/h per d ($R^2 = 0.32$).

There was a nonsignificant increase in feed consumption as the level of Ile in the diet was increased (Table 2). Body weight gain was significantly less when the hens received the diet with 0.49% Ile than BW gain of hens receiving all other diets, except for hens receiving Diet 5 with 0.52% Ile (Table 2).

The daily intake of Ile increased as the Ile content of the diet increased (Table 2), which was primarily due to increased Ile in the diet. However, a portion of the increase was due to the increased feed intake as the Ile content of the diet increased.

The Ile intake per gram of EC gradually increased as the Ile content increased. This increase was expected because it has been reported that the amount of EC increases the amount of Met (Harms et al. 1997) or Trp (Russell and Harms, 1999) required to produce a gram of EC.

The daily requirement of Ile in the present experiment was 589.2 mg for EP, 601.2 mg for EW, and 601.3 for EC. The requirement of 601 mg per day for 47.8 g EC would indicate 12.6 mg Ile was required for 1 g of EC. If the hen had produced 50 g EC, 630 mg Ile per hen per day would have been required, which is more than 472 mg as suggested by Bray (1969) and 573 mg as suggested by Harms and Ivey (1993), but is less than 650 mg suggested by NRC (1994), 660 mg by Schutte (1998), and 681 (579 mg digestible \div 85) suggested by Coon and Zhang (1999).

Addition of 10% more supplemental AA to the diet containing 0.52 and 0.55% Ile did not significantly affect EP, EW, or EC. The average EC for the two diets with

regular levels of AA was 46.50 g as compared with 46.55 g when the AA was added. This comparison indicated that Ile was first-limiting in this assay as designed.

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