

# Tryptophan requirement of first-cycle commercial laying hens in peak egg production

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**ABSTRACT** An experiment was conducted to evaluate the digestible tryptophan (Trp) requirement of laying hens from 22 to 34 wk of age. A total of 252 Hy-line W-36 laying hens were selected at 16 wk of age and allocated by weight ( $P = 0.90$ ) to 7 dietary treatments resulting in 12 replicate cages of 3 birds for each treatment. A Trp-deficient basal diet was formulated using corn, corn gluten meal, and soybean meal for each of the 3 dietary phases and supplemented with synthetic L-Trp to provide 105, 119, 133, 147, 162, 176, and 190 mg digestible Trp on a daily basis over the experimental period. To adapt the hens to experimental diets, pullets were fed complete diets that contained increasing amounts of corn gluten meal. Hens received a controlled amount of feed daily based on feed intake expected under

commercial conditions. Linear and quadratic broken-line, and quadratic polynomial models were used to estimate digestible Trp requirements based on hen-housed egg production (HHEP), egg mass (EM), and feed efficiency (FE). FE was calculated using EM and feed intake. Digestible Trp requirements were estimated to be 137, 183, and 192 mg/d for HHEP; 133, 180, and 183 for EM and 133, 177, and 173 for FE using linear broken-line, quadratic broken-line, and quadratic polynomial analysis, respectively. The quadratic broken line model in this experiment resulted in the best fit ( $R^2$ ) for all parameters measured. Linear broken line estimates resulted in lower estimates than the other models, and HHEP resulted in higher estimated digestible Trp requirement than EM and FE.

**Key words:** digestible tryptophan, egg production, laying hen, requirement, ratio

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## INTRODUCTION

Tryptophan (Trp) is an essential amino acid and third limiting in typical commercial corn-soybean diets for white egg-laying hens (Russell and Harms, 1999). Tryptophan is primarily used for protein synthesis to maintain egg production but also plays a role as a precursor of serotonin which is synthesized to melatonin to regulate sleep, appetite, and stress responses. In addition, Trp can be converted to the B-vitamin niacin through the kynurenine pathway (Rogers and Pesti, 1990). Genetic selection has resulted in annual improvement in egg production of 2 to 3 eggs per year and feed efficiency (1 to 2 point reduction in FE) in laying hens from 2010 to 2015 (Preisinger, 2018). This year over year improvement in productivity requires the reevaluation of amino acids requirements on an ongoing basis to support the

improved performance. Russell and Harms (1999) used 53- to 59-week-old Hy-Line W-36 laying hens to estimate a 136.5 mg/d Trp requirement when hens consumed 97 g/d of feed. Using younger 28- to 36-week-old Hy-Line W-36 laying hens, the same group estimated a 149 mg/d Trp requirement with 95 g of daily feed intake. More recently, 41- to 60-week-old Hy-Line W-36 laying hens were estimated to have a 153 mg/d Trp requirement when the hens consumed 97 g/d of feed (Wen et al., 2019). Several other authors have estimated requirements of laying hens using the Trp:Lysine ratio applying the ideal protein concept. Bregendahl et al. (2008) reported a Trp requirement of 120 mg/d of Trp relative to 482 mg/d of Lys based on broken line estimates using Hy-Line W-36 hens from 28 to 34 wk of age. A more recent publication estimated a higher requirement of 151 mg Trp/d relative to 675 mg/d of Lys based on 95 g/d intake of Hy-Line W-36 hens from 30 to 36 wk of age (Mousavi et al., 2017). However, none of the previous experiments have investigated the requirement of Trp as the pullets are transitioning into egg production. Therefore, the objective of the current experiment was to determine the Trp requirement of first cycle laying hens from 22 to 34 wk of age based on

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hen-housed egg production (HHEP), egg mass, and feed efficiency using linear broken line, quadratic broken line, and quadratic polynomial models.

## MATERIALS AND METHODS

### *Diet Formulation*

The Trp-containing ingredients (i.e., corn, soybean meal, and corn gluten meal) were analyzed for amino acid concentrations by wet chemistry (AOAC method 982.03, Eurofins, Des Moines, IA, 50321) before dietary formulation. Up to 7% corn gluten meal was used to replace soybean meal in the diet to generate the Trp-deficient diets. Low concentrations of 2 to 4% corn gluten meal were fed gradually starting in the pullet phase to slowly acclimate the pullets/hens and reduce any potential reductions in feed intake due to transitioning to experimental diets. All experimental diets were mixed using a basal diet that was analyzed for Trp (AOAC method #988.15, Eurofins, Des Moines, IA, 50321) before final experimental diet generation. The basal diet formulations were formulated to be adequate in all essential nutrients with the exception of Trp (Tables 1 and 2; Hy-Line International, 2018). The basal diets were split into equal aliquots to provide laying hens 105, 119, 133, 147, 161, 175, and 190 mg/d of digestible Trp via supplementation with Synthetic L-Trp (98% feed grade, CJ Bio, PT. Cheil Jedang Indonesia). Diets were formulated and mixed every 2 wk to meet the nutrient demands of the hens based on their feed consumption at each time point which increased up to 95 g/d intake. Diets were formulated and adjusted to maintain the same milligrams per day of Trp intake regardless of feed intake (Table 3). At the conclusion of the experiment, pooled samples of experimental diets were sent to Eurofins (Des Moines, IA, 50321) for amino acid analysis.

### *Animals and Housing*

All animal work was approved by the Institutional Animal Care and Use Committee. In total, 252 Hy-Line W-36 laying hen pullets were selected at 16 wk of age from a pullet flock of 500 hens and allocated to cages to have similar cage body weight (BW 1,532; SEM = 10;  $P = 0.90$ ). The 7 dietary treatments were randomly assigned to cages resulting in 12 replicate cages of 3 hens/cage (464.5 cm<sup>2</sup>/cage) utilizing 2 A-frame battery cage units. An environmentally controlled dark-out room was used to house birds. The temperature was set to be maintained between 68 and 75°F throughout the duration of the experiment although owing to summer heat conditions, temperatures reached a maximum 80°F for short periods (up to 4 h afternoon time periods). Hens were provided a controlled amount of feed of 85, 90, 95 g/hen/d from 22 to 24, 24 to 26, 26 to 34 wk of age, respectively. Hens were provided ad libitum access to water throughout the experiment. Experimental diets were provided starting at 16 wk of age to pretest hens

before data analysis began at 22 wk of age when consistent egg production was initiated.

Laying hens were monitored twice daily throughout the duration of the experiment and mortality and culled birds were noted, weighed, euthanized, and removed as they occurred. Feed allocation for the 2-wk period was weighed into individual labeled feed buckets for each replicate cage and daily allocation was provided at approximately 9 am from each individual feeding bucket. At the end of the 2-wk period, any remaining feed in the feed tray was returned to the corresponding feed bucket and weighed to determine feed intake. Eggs were counted, weighed, and collected by cage between 09:30 and 10:30 daily. Hen-housed egg production was calculated based on the number of eggs produced in relationship to the number of hens in each cage at the start of the experiment expressed on a percentage basis. Egg mass was calculated from egg weight and HHEP. Feed efficiency was calculated using daily EM divided by daily feed intake. Hens were weighed by cage every 4 wk.

### *Statistical Analysis*

Performance data, which included HHEP, feed intake, egg weight, egg mass, body weight, and feed efficiency were analyzed using a one-way ANOVA with means separated by repeated measures using a Tukey's adjustment in JMP 14 (SAS Institute Inc., Cary, NC; SAS Institute Inc., 2017). Significance was accepted at  $P \leq 0.05$ . Digestible Trp intake per hen was calculated from the feed intake and analyzed Trp values. Analyzed total Trp values were converted to digestible Trp values using AminoDat Software (Version 5, 2016) by multiplying the digestibility coefficient of each ingredient by the amount of Trp provided by that ingredient in the diet. These values were then used to determine the digestible Trp requirement for laying hens using linear broken line, quadratic polynomial, and quadratic broken line analyses for each of HHEP, EM, and FE. Regression analysis was performed using the nonlinear model analysis in JMP 14 (SAS Institute Inc., Cary, NC; SAS Institute Inc., 2017).

## RESULTS AND DISCUSSION

### *Laying Hen Performance*

Egg production ranged from 86.2 to 93.9% and generally increased until 147.5 mg digestible Trp after which the data appeared to plateau (Table 4). Overall mortality was negligible, with one mortality from hens fed 133.00 mg D Trp/d. Although there were no statistical differences among diets of 148 mg/d and above, there was a 1.7 percentage point increase in egg production. These results are consistent with previous research (Harms and Russell, 2000; Wen et al., 2019), whereas others found that egg production followed a quadratic pattern where egg production began to decrease at 0.19 and 0.20% or 210 mg/d and 181 mg/d, respectively

**Table 1.** Formulation of Trp-deficient basal diets fed to 22- to 34-week-old Hy-Line W-36 laying hens.

Ingredient	Peak	Peak	Peak
	22 to 24 wk of age	24 to 26 wk of age	26 to 34 wk of age
	(%)		
Corn	68.19	68.29	70.92
Corn gluten meal	7.00	7.00	7.00
Soybean meal	11.00	11.00	8.83
Soy oil	0.70	0.70	0.32
Salt	0.20	0.20	0.20
Sodium bicarbonate	0.13	0.13	0.13
L-Methionine	0.33	0.30	0.30
L-Lysine·HCl	0.57	0.50	0.51
L-Threonine	0.21	0.21	0.20
L-Valine	0.26	0.26	0.22
L-Isoleucine	0.23	0.23	0.23
L-Tryptophan	0.00	0.00	0.00
L-Arginine	0.32	0.32	0.27
Oyster shell (large particle)	4.69	4.69	4.69
Limestone (small particle)	4.69	4.69	4.69
Dicalcium phosphate	0.87	0.87	0.88
Phytase <sup>1</sup>	0.01	0.01	0.01
Choline chloride (60% choline)	0.10	0.10	0.10
Vitamin premix <sup>2</sup>	0.50	0.50	0.50
Total	100.00	100.00	100.00

<sup>1</sup>Quantum Blue (300 FTU/kg) was formulated to provide 0.12% of calcium and nonphytate phosphorus.

<sup>2</sup>Provided per kilogram of diet: vitamin A, 6,595 IU; vitamin D<sub>3</sub>, 2,209 ICU; vitamin E, 1.65 IU; vitamin B<sub>12</sub>, 6.60 µg; menadione, 1.15 mg; riboflavin, 4.12 mg; D-pantoic acid, 6.07 mg; niacin, 19.79 mg; choline, 381.68 mg; Co, 0.25 mg; Cu, 4.04 mg; I, 1.00 mg; Fe, 50.65 mg; Mn, 64.26 mg; Zn, 48.69 mg.

**Table 2.** Nutrient profile of Trp-deficient basal diets fed to 22- to 34-week-old Hy-Line W-36 laying hens.<sup>1, 2</sup>

Nutrient	Peak (22-24 wk of age)		Peak (24-26 wk of age)		Peak (26-34 wk of age)	
	Calculated	Analyzed	Calculated	Analyzed	Calculated	Analyzed
	(%)	(%)	(%)	(%)	(%)	(%)
Crude protein	16.19	16.18	16.20	16.05	15.30	15.45
ME, kcal/kg	2,954	—	2,954	—	2,951	—
Calcium	4.02	—	4.02	—	4.02	4.03
Nonphytate P	0.42	—	0.42	—	0.42	0.33
Fat	3.70	—	3.70	—	3.42	3.93
Met	0.61 (0.59)	0.56	0.58 (0.56)	0.60	0.58 (0.55)	0.54
Cys	0.26 (0.22)	0.24	0.26 (0.22)	0.25	0.26 (0.21)	0.23
Met + Cys	0.88 (0.82)	0.80	0.85 (0.79)	0.85	0.85 (0.77)	0.77
Lys	1.03 (0.96)	1.04	0.98 (0.91)	1.04	0.98 (0.86)	0.97
His	0.39 (0.29)	0.38	0.39 (0.29)	0.41	0.39 (0.28)	0.37
Trp	0.15 (0.12)	0.16	0.15 (0.12)	0.16	0.15 (0.11)	0.14
Thr	0.78 (0.69)	0.74	0.78 (0.69)	0.78	0.78 (0.66)	0.71
Arg	1.08 (1.01)	0.98	1.08 (1.01)	1.05	1.08 (0.90)	0.87
Ile	0.81 (0.76)	0.77	0.80 (0.76)	0.85	0.81 (0.73)	0.75
Leu	1.79 (1.65)	1.75	1.79 (1.65)	1.87	1.79 (1.60)	1.70
Val	0.94 (0.87)	0.91	0.94 (0.87)	0.99	0.90 (0.80)	0.86
Gly	0.55 (0.50)	0.55	0.55 (0.50)	0.59	0.55 (0.47)	0.52
Ser	0.77 (0.54)	0.77	0.77 (0.54)	0.81	0.77 (0.54)	0.68

<sup>1</sup>Diets were formulated to the same nutrient intake on a daily basis and differences in formulation were to account for feed intake. Basal and experimental diets were mixed every 2 wk and analysis of basal diets was on a pooled basis if diets were fed for more than 2 wk.

<sup>2</sup>Calculated amino acid values are reported as total dietary amino acid (digestible amino acid) and analyzed amino acid values are reported as total dietary amino acid.

(Jensen et al., 1990; Cardoso et al., 2014). As expected, low digestible Trp diets had a 7.7 percentage point reduction in egg production compared with the diets with the highest dietary digestible Trp concentration. Average BW ranged from 1,481 to 1,536 g which is consistent with the Hy-Line W-36 performance objectives for this age. There was a tendency ( $P = 0.06$ ) for a dose response of Trp concentration on BW as the hens fed the lowest concentrations of Trp had the lowest BW. In a previous report, Wen et al. (2019) reported a quadratic response to BW loss where the BW loss was lowest with the 169 mg/d inclusion of Trp, then increased with additional supplementation. However, when laying hens were fed diets containing 145 mg/d Trp or above (Trp:Lys ratio of 17), there was no observed BW loss (Bregendahl et al., 2008). These differences might be due to differences in time of experiments

as the longer experiments would have more time for differences in BW to manifest. Feed intake over the 22- to 34-wk period ranged from 91.7 to 93.1 g/d. Overall there were few differences across the range of digestible Trp diets (feed intake across the 7 treatment diets were generally within 1 g). Differences in feed intake among treatments were only observed between the lowest 2 concentrations of Trp where hens fed 119 mg/d had higher feed intake than those fed 105 mg/d. These data are inconsistent with previous reports that found feed intake was reduced with deficient concentrations of Trp (Russell and Harms, 1999; Wen et al., 2019).

Although Trp in the diet had a significant effect on the egg weight ( $P \leq 0.01$ ), no clear pattern was observed, and the biological relevance of this response is questionable. The literature is also inconsistent regarding the effect of Trp on egg weights with reports of a dose response

**Table 3.** Formulated and analyzed digestible Trp content of experimental diets fed to 22- to 34-wk old laying hens.<sup>1</sup>

Digestible Trp Mg/d	Peak (22-24 wk of age)		Peak (24-26 wk of age)		Peak (26-34 wk of age)	
	Calculated <sup>2</sup>	Analyzed <sup>2</sup>	Calculated <sup>2</sup>	Analyzed <sup>2</sup>	Calculated <sup>2</sup>	Analyzed <sup>2</sup>
	(%)	(%)	(%)	(%)	(%)	(%)
105	0.148 (0.123)	0.16 (0.13)	0.149 (0.116)	0.15 (0.12)	0.136 (0.110)	0.14 (0.12)
119	0.165 (0.140)	—	0.157 (0.132)	0.16 (0.13)	0.155 (0.125)	0.16 (0.13)
133	0.181 (0.156)	0.17 (0.14)	0.173 (0.148)	—	0.165 (0.140)	0.16 (0.13)
147	0.198 (0.173)	—	0.189 (0.164)	0.18 (0.15)	0.185 (0.155)	0.19 (0.16)
162	0.215 (0.190)	0.20 (0.17)	0.204 (0.179)	—	0.198 (0.170)	0.20 (0.17)
176	0.232 (0.207)	—	0.220 (0.195)	0.21 (0.17)	0.209 (0.185)	0.20 (0.17)
190	0.249 (0.224)	0.22 (0.18)	0.236 (0.211)	—	0.226 (0.200)	0.22 (0.18)

<sup>1</sup>Supplemental L-Trp was added to the basal diet to generate final experimental diets that contained 104.50, 118.75, 133.00, 147.25, 161.50, 175.75, and 190.00 mg per day of digestible Trp based on projected feed intake.

<sup>2</sup>Calculated amino acid values are reported as total dietary amino acid (digestible amino acid) and analyzed amino acid values are reported as total dietary amino acid (digestible amino acid).

**Table 4.** Body weight (BW), hen-housed egg production (HHEP), feed intake (FI), egg weight (EW), egg mass (EM) and feed efficiency (FE) of 22- to 34-week-old laying hens fed 104.50, 118.75, 133.00, 147.25, 161.50, 175.75, and 190.00 mg digestible Trp per day.<sup>1</sup>

Dietary Trp (mg/d)	BW <sup>2</sup> (g)	HHEP <sup>3</sup> (%)	FI (g/d)	EW (g)	EM (g)	FE (g:kg)
105	1,481	86.3 <sup>c</sup>	91.7 <sup>b</sup>	54.3 <sup>a</sup>	46.7 <sup>b</sup>	510 <sup>c</sup>
119	1,489	90.8 <sup>a,b</sup>	93.2 <sup>a</sup>	53.4 <sup>b</sup>	48.4 <sup>a,b</sup>	519 <sup>b,c</sup>
133	1,495	89.1 <sup>b,c</sup>	92.7 <sup>a,b</sup>	54.3 <sup>a</sup>	49.8 <sup>a</sup>	531 <sup>a,b</sup>
147	1,515	92.3 <sup>a,b</sup>	92.7 <sup>a,b</sup>	53.4 <sup>b</sup>	49.2 <sup>a</sup>	538 <sup>a</sup>
162	1,519	92.6 <sup>a</sup>	92.9 <sup>a,b</sup>	54.0 <sup>a,b</sup>	50.2 <sup>a</sup>	539 <sup>a</sup>
176	1,536	93.7 <sup>a</sup>	92.5 <sup>a,b</sup>	54.6 <sup>a</sup>	49.9 <sup>a</sup>	540 <sup>a</sup>
190	1,517	94.0 <sup>a</sup>	92.6 <sup>a,b</sup>	53.2 <sup>b</sup>	50.0 <sup>a</sup>	540 <sup>a</sup>
Pooled SEM	43	0.79	0.28	0.32	0.53	4.5
ANOVA	<i>P</i> value					
Treatment	0.06	≤ 0.01	0.01	≤ 0.01	≤ 0.01	≤ 0.01
Week	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Trt x Week	0.67	0.99	0.98	0.99	0.99	0.99

<sup>a-c</sup>Means within a column that do not share a common superscript differ ( $P \leq 0.05$ ).  
<sup>1</sup>Initial BW = 1,532 g ( $P = 0.65$ ).  
<sup>2</sup>BW measurement was based on a repeated measure every 4 wk throughout the experimental period.  
<sup>3</sup>Overall mortality was negligible, with one mortality from hens fed 133.00 mg D Trp/d.

effect on egg weights (Russell and Harms, 1999; Wen et al., 2019) and other reports (Jensen et al., 1990; Cardoso et al., 2014) without differences in egg weights with various dietary Trp concentrations.

Egg mass values ranged from 46.7 to 50.2 g, which is consistent with breed objectives at this age. Egg mass and feed efficiency responded similarly as both increased until approximately the 162 mg/d Trp treatments with no additional improvements above that treatment. These results are generally consistent with egg mass and FE results from previous reports (Bregendahl et al., 2008; Cardoso et al., 2014; Wen et al., 2019).

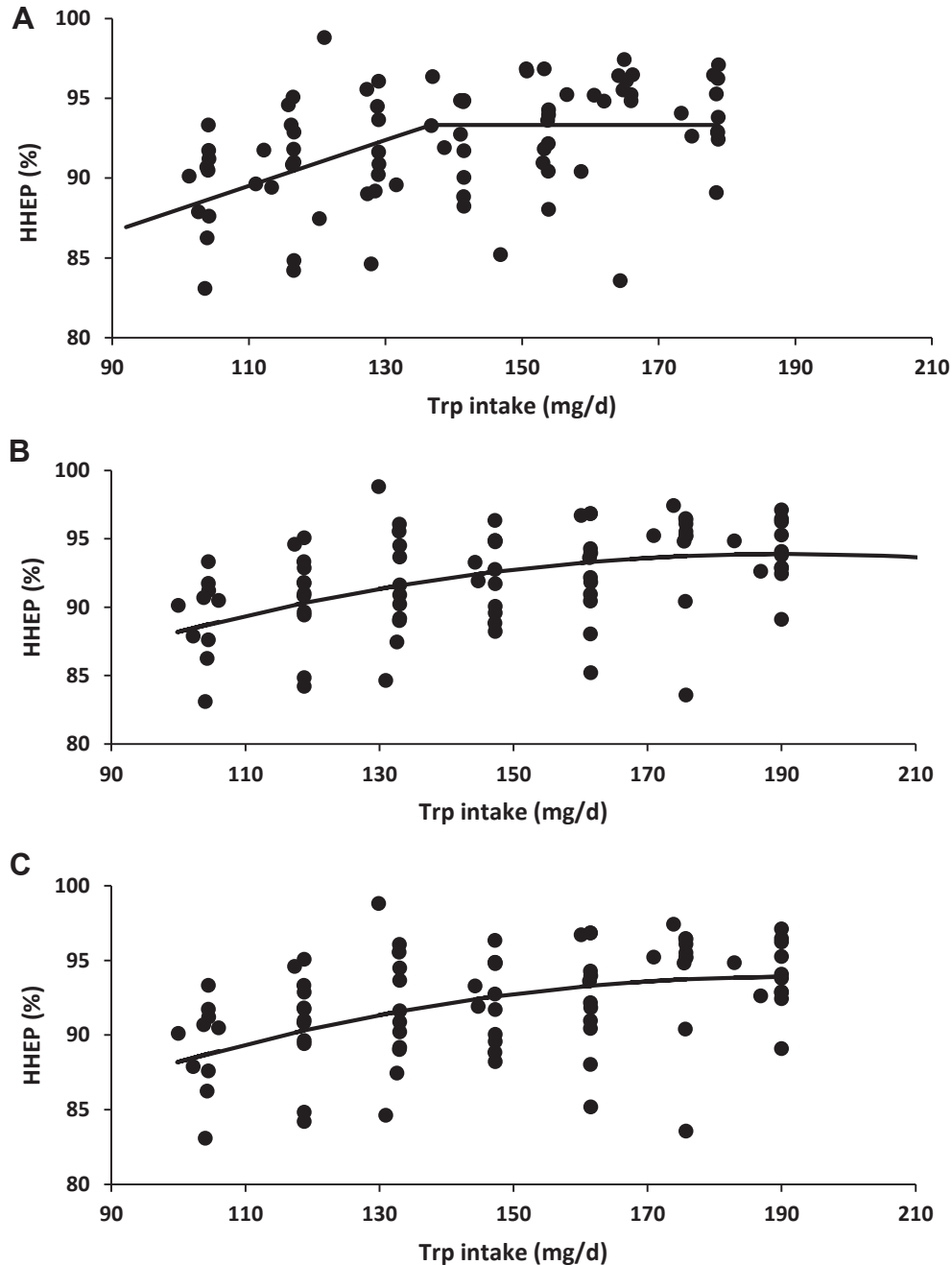
### Tryptophan Requirement

The linear broken line model resulted in estimates of 137, 133, and 133 mg/d of digestible Trp for HHEP, egg mass, and feed efficiency, respectively (Table 5; Figure 1). The linear broken line analysis is the classic method of modeling requirements of amino acids defined by Morris and Jennings (1973). The model is based on an increasing or decreasing slope portion where the response is increasing with every increasing supplementation of a

nutrient because the animal is below requirement for that specific nutrient; however, after the animal reaches the requirement, the result is a straight-line plateau without a slope. The point at which that plateau starts, or the line breaks is the maximum response and requirement estimate. In this experiment, the linear broken line method estimated the lowest Trp requirements compared with the other models which is consistent with previous estimations of amino acid requirements in the literature (Morris and Jennings, 1973; Wen et al., 2019). Russell and Harms (1999) found the requirement for Trp to be 136 mg/d of total Trp or 107 mg/d of digestible Trp for Hy-line W-36 laying hens from 53 to 59 wk of age. In another study, Harms and Russell (2000) found the requirement for HHEP to be 140 mg/d of total Trp which would be estimated to 110 mg/d of Digestible Trp for Hy-line 36 laying hens from 28 to 36 wk of age. Finally, the most recent experiment investigating Trp requirement in Hy-line W-36 hens from 41 to 60 wks of age determined a requirement of 153, 156, and 140 mg/d of total Trp for HHEP, egg mass, and FE (Wen et al., 2019). These requirements can be converted to 120, 122, and 110 mg/d of digestible

**Table 5.** Trp requirement (mg/d) of 22- to 34-week-old Hy-Line laying hens estimated using linear broken-line, quadratic broken-line, and quadratic polynomial models based on hen-housed egg production (HHEP), egg mass (EM) and feed efficiency (FE).

Response variables	Model	Trp requirement	Ordinate value	Regression equation	R <sup>2</sup>
HHEP (%)	Linear broken line	137	93.3	$y = 93.3 - 0.1(x - 136.6)$	0.83
	Quadratic broken line	183	93.5	$y = 93.5 - 0.0009(x - 183.3)^2$	0.96
	Quadratic polynomial	192	93.4	$y = 61.7 + 0.3x - 0.0009x^2$	0.78
EM (g/d)	Linear broken line	133	50.1	$Y = 50.2 - 0.1(x - 133.0)$	0.77
	Quadratic broken line	180	51.2	$y = 51.2 - 0.0004(x - 180.0)^2$	0.96
	Quadratic polynomial	183	51.2	$y = 32.3 + 0.2x - 0.0006x^2$	0.84
FE (g/kg)	Linear broken line	133	540.0	$y = 540.0 - 0.8(x - 132.5)$	0.81
	Quadratic broken line	177	543.8	$y = 543.8 - 0.006(x - 177.4)^2$	0.96
	Quadratic polynomial	173	541.6	$y = 386.4 + 1.7x - 0.005x^2$	0.82



**Figure 1.** Digestible Trp requirement of 22- to 34-week-old Hy-Line W-36 laying hens estimated using (A) linear broken line regression;  $y = 93.3 - 0.1(x - 136.6)$ ;  $R^2 = 0.83$ , (B) quadratic polynomial regression;  $y = 61.7 + 0.3x - 0.0009x^2$ ;  $R^2 = 0.78$ , and (C) quadratic broken line regression;  $y = 93.5 - 0.0009(x - 183.3)^2$ ;  $R^2 = 0.96$  for hen-housed egg production (HHEP; %).

Trp. Contrasting historical results with current findings, the Trp requirement for laying hens coming into production might be higher to support the peak production of laying hens when egg production is at the highest output and efficiency. In addition, Trp is needed along with other amino acids to support the growth and development of hens to sustain and support egg production for longer periods.

Although the linear broken-line model is the classical requirement method, others have however contended that the idea is not to formulate to a requirement for maximum performance but instead to provide the

nutrients for a maximum profit (Pesti et al., 2009). Therefore, a variety of models can be used to estimate further responses that the linear broken line does not incorporate. Quadratic polynomial analysis has also been widely used to estimate requirements based on the concept of “diminishing marginal productivity” where the effect of every addition of a nutrient is diminished until you reach the requirement (Pesti et al., 2009). However, the issue that is faced with quadratic polynomial is that it lacks a clear plateau for determining requirements and after the maximum response is achieved, the response falls rapidly which is not

**Table 6.** Effects of laying hen feed intake on Trp requirement when gelatin or corn gluten meal is introduced into Trp-sufficient experimental diets with or without an adaptation period.

Reference	Adapted	Time	Feed intake	Expected feed intake
		(Weeks)	(g)	(g)
Russell and Harms (1999)	No	53 to 59	82-86	97
Harms and Russell (2000)	No	28 to 36	90	95
Wen et al. (2019)	No	41 to 47	80-86	97
Current experiment	Yes	22 to 28	87	88

consistent with biological data. Furthermore, there are differences in where the requirement should be estimated as some reports have used 95% of the maximum response (Wen et al., 2019) whereas others (Pesti et al., 2009; Cardoso et al., 2014) have used the maximum response. The quadratic polynomial resulted in estimates of 192, 183, and 173 mg/d of digestible Trp at 95% of the maximum response for HHEP, egg mass, and feed efficiency, respectively. In Hy-line W-36 laying hens from 41 to 60 wk, the quadratic polynomial estimates were 180, 187, and 182 mg/d of total Trp or 142, 147, and 143 mg/d of digestible Trp for HHEP, egg mass, and FE, respectively (Wen et al., 2019). Other authors have explored modeling Trp requirements based on the ratio to lysine using the linear broken line (Bregendahl et al., 2008) or quadratic polynomial model (Lima et al., 2012; Cardoso et al., 2014). The digestible Lys intake of the laying hens in this experiment was 800 mg/d, consistent with previous Lys requirement estimates for laying hen egg production and egg mass, 727 mg/d to 846 mg/d (Pastore et al., 2018; Spangler et al., 2019). The estimates for Trp:Lys ratio in this experiment using the linear broken line were 17.1, 16.6, and 16.6 for egg production, egg mass, and feed efficiency, respectively. This estimate is lower than the previous report of 24.6, 24.5, and 22.3 Trp:Lys ratios found in Hy-Line W-36 laying hens from 28 to 34 wk of age (Bregendahl et al., 2008). Estimates from the quadratic polynomial were 24.0, 22.9, and 21.6 for egg production, egg mass and feed efficiency, respectively. These results are in agreement with previous reports of estimated requirements of 23.9 to 25.4 for egg production, egg mass, and feed efficiency (Lima et al., 2012; Cardoso et al., 2014).

The quadratic broken line includes the advantages of a plateau region of the linear broken line and the diminishing marginal productively of the quadratic polynomial. The requirement can be estimated at the point where the line breaks in the same manner as the linear broken line analysis (Pesti et al., 2009). The estimates based on the quadratic broken line were 183, 180, and 177 mg/d of digestible Trp for HHEP, egg mass, and feed efficiency, respectively. These estimates were higher than the estimates for the linear broken line, but they were slightly lower or similar to quadratic polynomial estimates. In comparison, Wen et al. (2019) found the requirement of total Trp to be 182, 200, and 164 mg/d or 143, 157, and 129 mg/d of digestible Trp for HHEP, egg mass,

and FE. Overall, results from all models indicate that laying hens coming into production have a higher requirement for Trp to satisfy the requirements of egg production while maintaining and possibly increasing their body weight. Furthermore, there seems to have been an increase in Trp requirements due to genetic selection that can be observed when comparing these results with the findings of Russell and Harms (1999).

In previous Trp requirement studies where gelatin or corn gluten meal were used to generate Trp-deficient diets, there was an associated drop in feed intake after the transition to experimental diets in the diets that were formulated to be sufficient in dietary Trp (Table 6). The introduction of gelatin to experimental diets caused feed intake to drop down to 82-86 g compared with an expected intake of 97 g/d (Russell and Harms, 1999). Corn gluten meal in experimental diets without an adaptation period also resulted in a drop of 11 to 17 g/d drop in feed intake in the first 6 wk (Wen et al., 2019). However, the current experiment resulted in no reduced feed intake in Trp sufficient diets after the transition to experimental diets. This was largely due to the adaptation of pullets to experimental diets by adding corn gluten meal in stages during the pullet phase of production.

The current experiment indicated a higher Trp requirement than previously seen in the literature as the hens are transitioning into egg production to support the increased egg production up to peak. The quadratic broken line model in this experiment resulted in the best fit ( $R^2$ ) for all parameters measured compared with the other 2 models. The adaptation period before the start of this experiment allowed for a more accurate estimation of requirements because no negative effects on feed intake were observed in the transition to experimental diets. For maximizing egg production, the optimum intake of digestible Trp was 137, 183, and 192 based on the linear broken line, quadratic broken line, and quadratic polynomial, respectively.

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## DISCLOSURES

Albaraa Sarsour and Mike Persia declare no conflict of interest. Jason Lee and Keith Haydon both work for the sponsoring company, but were not involved in data collection or analysis; their contributions came from experimental design and review of the manuscript.

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