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Effect of Amino Acid Formulation and Dietary Direct-Fed Microbial Supplementation on Egg Production and Egg Characteristics in Laying Hens

Effect of amino acid formulation and dietary direct-fed microbial supplementation on egg production and egg characteristics in laying hens

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Primary Audience: Nutritionists, Researchers, Veterinarians, Flock Supervisors

SUMMARY

An experiment was conducted to determine whether direct-fed microbial supplementation could alleviate a marginal amino acid (AA) deficiency in Hyline 36 laying hens from 33 to 44 wk of age. The experiment was a 2 × 4 factorial design with or without a commercial direct-fed microbial (Primilac; 1.36 kg/1,000 kg) and 4 levels of AA formulation. Egg characteristics (yolk, albumen, or shell proportions and yolk or albumen solids) were not affected by diet. Primilac supplementation had no effect on egg production or egg mass. However, Primilac supplementation reduced feed intake-to-egg mass ratio by 2.4 and 3.4% from 33 to 36 wk and 41 to 44 wk, respectively. Total eggs laid and egg mass were greatest when at least 14.4 g of CP, 804 mg of Lys, 382 mg of Met, 601 mg of TSAA, 502 mg of Thr, and 609 mg of Ile were consumed per hen per day from 33 to 44 wk of age. In conclusion, Primilac supplementation was not able to completely alleviate a marginal AA deficiency in laying hens but did improve feed intake-to-egg mass ratios during 8 wk of the 12-wk study.

Key words: amino acid, direct-fed microbial, egg production, laying hen

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DESCRIPTION OF PROBLEM

Several reports have demonstrated that direct-fed microbials (**DFM**), or probiotics, improve the performance and efficiency of nutrient use in broilers [1–5] and in laying hens and pullets [6–12]. This improvement, as reviewed by Patterson and Burkholder [13], is a result of bacterial antagonism, competition for colonization sites, competition for nutrients, reduction in the production of toxic compounds, and stimulation of the immune system. The end result, in some cases, is an improved intestinal health that results in higher intestinal enzyme activities and nutrient absorption [6]. Work done with laying hens and broilers has demonstrated that the addition of DFM to the diet may improve N, Ca, and P retention levels [5, 6, 8].

Angel et al. [5] noted that when a diet containing 12% less CP, Lys, Met, and TSAA was fed

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to broilers, no performance was lost if a DFM was included in the diet compared with a nutrient-adequate control diet. Similarly, Nahashon et al. [8] reported improvements in laying hen performance when a DFM was supplemented in low CP, but essential amino acid (**AA**)-adequate, diets. This work demonstrates that the benefits of the DFM may have occurred in part through an improvement in nutrient availability. The report by Nahashon et al. [8] used a split-plot experimental design with laying hens fed a control dietary CP of 15.3% (with or without a lactobacillus-based DFM) or a CP of 14.3 or 13.3% supplemented with a lactobacillus-based DFM. The 14.3 and 13.3% CP groups were adequate in all AA [14] except Ile (calculated analyses = 628 and 603 mg/hen per day, respectively, compared with the NRC [14] recommendation of 650 mg/hen per day). Based on the data set of Nashashon et al. [8], a study with a complete factorial arrangement was conducted to determine 1) if a difference in performance response of Hy-Line W36 laying hens would exist when hens were fed a lower CP diet with DFM compared with a diet with a similar CP without DFM supplementation, and 2) if DFM supplementation would improve Hy-Line W36 performance between wk 33 to 44 when essential AA, other than Ile, were limiting.

MATERIALS AND METHODS

All research reported herein was approved by the Purdue University Animal Care and Use Committee.

A 2 × 4 factorial experiment was conducted with or without a commercial lactobacilli-based DFM (0.15%; Primilac) [15] and 4 concentrations of CP and AA. Three diets varying in CP and AA concentrations were formulated (adequate, marginal, and deficient; Table 1). The adequate AA diet was fed either ad libitum or at 90 g/hen per day; and the marginal and deficient diets were fed at 90 g/hen per day. The predicted CP and AA intakes based on a 90 g/hen per day of feed intake and the calculated dietary nutrient formulation are presented in Table 2. The commercial DFM, Primilac [15], contains, among others, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidium*, and *Enterococcus faecium*. The specifics of the blend concentrations are proprietary.

Basal mixes for each of the 3 diets varying in CP and AA concentration were mixed. Subsequently, Celite [16] was added at 1.5% to the basal diets for the diets with no DFM, and 1.35% Celite plus 0.15% of the DFM was added to the basal diets for the diets containing the DFM. Diets were fed in mash form. Diets without DFM were mixed before those with DFM

Table 1. Formulated diets and calculated and determined nutrient content for diets varying in CP and amino acid concentrations

	Diet[1]		
Item	Adequate	Marginal	Deficient
Ingredient, %			
Corn	56.04	58.57	60.43
Soybean meal, 48% CP	24.00	21.90	20.30
Soybean oil	6.00	5.64	5.40
NaCl	0.41	0.41	0.41
DL-Met	0.22	0.17	0.15
L-Lys HCl	0.07	0.05	0.05
Limestone	9.91	9.91	9.91
Dicalcium phosphate	1.50	1.50	1.50
Vitamin-mineral premix[2]	0.35	0.35	0.35
Celite	1.50	1.50	1.50
Calculated content, %			
ME_n, kcal/kg	3,000	3,000	3,000
CP	16.10	15.24	14.60
Lys	0.90	0.83	0.78
Met	0.47	0.42	0.39
TSAA	0.75	0.68	0.64
Thr	0.62	0.58	0.56
Ile	0.67	0.63	0.60
Ca	4.19	4.19	4.18
Total P	0.61	0.60	0.59
Nonphytate P	0.39	0.39	0.39
Determined content,[3] %			
CP	17.42	16.55	15.48
Lys	0.97	0.89	0.82
Met	0.46	0.45	0.35
TSAA	0.72	0.69	0.58
Thr	0.60	0.57	0.53
Ile	0.73	0.66	0.60

[1]The adequate diet was fed to 2 treatments of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day. A commercial direct-fed microbial (Primilac) [15] was added to diets at 0.15% at the expense of Celite (diatomaceous earth) [16].

[2]Supplied per kilogram of diet: vitamin A, 12,320 IU; vitamin D_3, 4,620 IU; vitamin E, 15.4 IU; vitamin K, 3.08 mg; riboflavin, 6.16 mg; niacin, 46.2 mg; vitamin B_{12}, 23.1 mg; pantothenic acid, 15.4 mg; folic acid, 0.31 mg; choline, 401 mg; iron, 50.4 mg; zinc, 71 mg; manganese, 90 mg; copper, 7 mg; iodine, 0.7 mg; and selenium, 0.25 mg.

[3]Determined nutrient content was conducted in duplicate for each of 3 mixing batches. Results presented within this table are averages of each diet at each mixing time.

Table 2. Calculated CP and amino acid intake of hens based on formulated nutrient concentrations

	Diet		
Nutrient	Adequate[1]	Marginal	Deficient
CP, g/hen per day	14.49	13.71	13.14
Lys, mg/hen per day	813	745	705
Met, mg/hen per day	426	374	349
Thr, mg/hen per day	554	524	502
Ile, mg/hen per day	604	569	542

[1]The adequate diet was fed to 2 treatment groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum; however, feed intake was less than 90 g/hen per day for all treatments (Table 3). The marginal and deficient diets were fed at 90 g/hen per day.

to minimize potential contamination with the DFM. Diets were mixed every 4 wk. All diets were formulated using a single batch of soybean meal analyzed for AA and CP content before the beginning of the study.

Hyline W36 hens [17] were used for this experiment. Each dietary treatment was fed to birds in 24 cages, with 2 birds per cage. Each hen had 780 cm^2 of cage-floor space. The room where the experiment was conducted had 2 separate entry doors. To minimize cross-contamination with the DFM, cages with DFM-fed birds were located on one-half of the room, whereas the birds not fed DFM were located on the other half [5, 18]. Treatments were randomized within each side of the room and blocked by cage level. Further care was taken to prevent DFM contamination of birds not fed DFM by 1) ensuring that staff wore clean foot covers, gloves, and laboratory coats; 2) washing floors daily into a central shallow pit; 3) collecting eggs, feeding birds, and weighing birds not fed the DFM first; and 4) storing diets separately. Each cage of birds was individually fed on a daily basis. For the birds receiving 90 g/hen per day, cups whose volumes

Table 3. Feed intake over a 3-d period of hens fed diets with or without Primilac[1] at varying CP and amino acid formulations[2]

		Weeks of age		
Primilac	CP level	36	40	44
		(g/hen per day)		
No	Adequate ad libitum[3]	86.7[a]	80.5[ab]	86.2[a]
No	Adequate	83.3[bc]	82.7[ab]	85.9[ab]
No	Marginal	81.4[c]	79.2[b]	78.0[c]
No	Deficient	83.5[bc]	80.7[ab]	81.9[bc]
Yes	Adequate ad libitum	84.9[ab]	81.9[ab]	80.4[c]
Yes	Adequate	82.9[bc]	83.6[a]	80.5[c]
Yes	Marginal	80.4[c]	84.3[a]	80.6[c]
Yes	Deficient	80.9[c]	83.2[ab]	79.8[c]
SEM		0.73	1.04	0.99
		Probability		
Source of variation				
Primilac		0.0057	0.002	0.0001
CP level		0.0001	0.55	0.002
Primilac × CP level		0.44	0.12	0.004
Main effect means				
No		83.7	80.8	83.5
Yes		82.3	83.1	80.3
	Adequate ad libitum	85.8[a]	81.8	83.3[a]
	Adequate	83.1[b]	82.7	83.2[a]
	Marginal	82.2[bc]	81.8	80.8[b]
	Deficient	80.9[c]	81.9	80.3[b]

[a–c]Means within a column with no common superscript differ as a result of Tukey means comparison ($P < 0.05$).

[1][15].

[2]Means represent 24 cages per diet, 2 birds per pen.

[3]The adequate diet was fed to 2 treatment groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day.

were adjusted to contain 180 g were used and the volume was specific for each diet.

Crude protein content of feed samples was determined, in duplicate, using a Leco model FP 2000 N combustion analyzer [19, 20]. Feed samples for AA analyses [21] were hydrolyzed in 6 *N* HCl for 24 h at 110°C under N atmosphere. Performic acid oxidation was carried out before acid hydrolysis for analysis of Met and Cys. Samples for Trp analysis were hydrolyzed using barium hydroxide [22]. Amino acids in the hydrolysate were subsequently determined by HPLC after postcolumn derivatization. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis. Diet AA content was analyzed in duplicate.

Egg production was recorded daily. Egg weight was determined from a 2-d egg collection on a weekly basis. Feed disappearance was determined over 3 consecutive days every 4 wk. Egg characteristics were determined every 4 wk from 2 consecutive days of egg collection. Egg characteristics included yolk, albumen, and shell contents as a percentage of total egg weight. Shell weight was determined after air-drying at room temperature for 24 h. Yolk and albumen solids contents were determined after freeze-drying.

Data were analyzed as a 2 (DFM supplementation) × 4 (CP and AA concentration) factorial experiment using the GLM procedure of SAS [23]. The interaction of DFM supplementation and dietary AA concentration was included in the model. A cage of hens was the experimental unit. Means separation was accomplished by Tukey's test and the level of significance was set at $P \leq 0.05$. Differences between treatments were considered significant when $P \leq 0.05$ unless stated otherwise.

RESULTS AND DISCUSSION

The determined contents of dietary AA were close to formulated values for the adequate,

Table 4. Average CP and amino acid intake of hens fed diets with or without Primilac[1] at varying CP and amino acid formulations[2]

Primilac	CP level	CP	Lys	Met	TSAA	Thr	Ile
		(g/hen per day)	(mg/hen per day)				
No	Adequate ad libitum[3]	14.6[a]	816.8[a]	387.8[a]	610.2[a]	510.2[a]	619.4[a]
No	Adequate	14.6[a]	813.5[a]	386.2[a]	607.7[a]	508.3[a]	616.9[a]
No	Marginal	13.5[b]	717.8[b]	361.4[c]	558.4[b]	461.5[b]	537.1[b]
No	Deficient	12.8[c]	669.7[c]	287.1[d]	475.7[c]	434.5[c]	494.8[c]
Yes	Adequate ad libitum	14.4[a]	796.2[a]	378.7[a]	595.6[a]	496.8[a]	603.9[a]
Yes	Adequate	14.3[a]	793.5[a]	377.6[ab]	593.8[a]	495.1[a]	601.9[a]
Yes	Marginal	13.6[b]	724.9[b]	365.1[bc]	564.0[b]	466.1[b]	542.4[b]
Yes	Deficient	12.7[c]	663.9[c]	284.6[d]	471.5[c]	430.9[c]	490.3[c]
SEM		0.11	6.40	3.09	4.83	4.04	4.84
		Probability					
Source of variation							
Primilac		0.18	0.03	0.06	0.049	0.03	0.03
CP level		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Primilac × CP level		0.22	0.10	0.13	0.13	0.08	0.10
Main effect means							
No		13.8	754.4	355.6	563.0	478.7	567.1
Yes		13.7	744.6	351.5	556.2	472.2	559.6
	Adequate ad libitum	14.5[a]	806.5[a]	383.2[a]	602.9[a]	503.5[a]	611.6[a]
	Adequate	14.4[a]	803.5[a]	381.9[a]	600.7[a]	501.7[a]	609.4[a]
	Marginal	13.5[b]	721.3[b]	363.2[b]	561.2[b]	463.8[b]	539.8[b]
	Deficient	12.7[c]	666.8[c]	258.8[c]	473.6[c]	432.7[c]	492.6[c]

[a–d]Means within a column with no common superscript differ as a result of Tukey means comparison ($P < 0.05$).

[1][15].

[2]Means represent 24 cages per diet, 2 birds per pen.

[3]The adequate diet was fed to 2 treatment groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day.

Table 5. Hen BW when fed diets with or without Primilac[1] at varying CP and amino acid formulations[2]

		Weeks of age			
Primilac	CP level	33	37	41	44
		(kg/hen)			
No	Adequate ad libitum[3]	1.60	1.55^{a}	1.58^{a}	1.56^{ab}
No	Adequate	1.57	1.51^{ab}	1.53^{abc}	1.52^{bc}
No	Marginal	1.58	1.48^{abc}	1.47^{bcd}	1.45^{cd}
No	Deficient	1.57	1.48^{abc}	1.45^{cd}	1.43^{d}
Yes	Adequate ad libitum	1.51	1.51^{ab}	1.54^{ab}	1.61^{a}
Yes	Adequate	1.52	1.47^{bc}	1.47^{bcd}	1.46^{cd}
Yes	Marginal	1.52	1.47^{bc}	1.44^{d}	1.45^{cd}
Yes	Deficient	1.52	1.43^{c}	1.43^{d}	1.42^{d}
SEM		0.022	0.017	0.019	0.017
		Probability			
Source of variation					
Primilac		0.0001	0.003	0.002	0.57
CP level		0.91	0.002	0.0001	0.001
Primilac × CP level		0.73	0.76	0.69	0.02
Main effect means					
No		1.58	1.50	1.51	1.49
Yes		1.52	1.47	1.47	1.49
	Adequate ad libitum	1.56	1.53^{a}	1.56^{a}	1.59^{a}
	Adequate	1.54	1.49^{ab}	1.50^{b}	1.49^{b}
	Marginal	1.55	1.47^{b}	1.46^{bc}	1.45^{bc}
	Deficient	1.54	1.46^{b}	1.44^{c}	1.42^{c}

$^{a–d}$Means within a column with no common superscript differ as a result of Tukey means comparison ($P < 0.05$).

[1][15].

[2]Means represent 24 cages per diet, 2 birds per pen.

[3]The adequate diet was fed to 2 treatment groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day.

marginal, and deficient diets. However, the CP was higher than formulated (1.3, 1.3, and 0.9 percentage units for the adequate, marginal, and deficient diets, respectively; Table 1). Because feed intakes (Table 3) were generally lower than predicted (Table 2), the differences in CP from formulated values brought the actual intakes of CP (Table 4) close to predicted levels.

Feed intake, as determined over 3 d in each 4-wk period, is presented in Table 3. Feed intake was reduced when Primilac was added, by 1.7 and 3.8% at 36 and 44 wk of age, respectively, but was increased by 2.8% at 40 wk of age. Feeding more CP and AA tended to increase feed consumption at 36 and 44 wk of age, but not at 40 wk of age. At 44 wk of age, there was a Primilac × CP concentration interaction. Providing ad libitum access to the adequate AA diet (without Primilac) or the same diet at 90 g/hen per day resulted in the highest feed intake when compared with any other treatment. As expected, hens consuming adequate CP and AA concentrations had greater average daily intakes of CP, Lys, Met, TSAA, Thr, and Ile when compared with birds fed the marginal or deficient CP and AA diets (Table 4). Birds supplemented with Primilac consumed 10, 4, 7, 6.5, and 7.5 mg of Lys, Met, TSAA, Thr, and Ile less per hen per day on average throughout the study.

Hen BW was determined before and after each 4-wk period and are presented in Table 5. The Primilac-supplemented hens were 60 g lighter at the beginning of the experiment (33 wk of age) but were not different at 44 wk of age. Notably, hens supplemented with Primilac lost only 2% of their initial BW over the 12-wk laying period, whereas unsupplemented hens lost 6% of their initial BW. Feeding less AA and CP caused the hens to lose more BW over the course of the experiment, such that hens fed the deficient AA diet lost 8% of BW during the 12-wk laying period.

Table 6. Number of eggs from hens fed diets with or without Primilac[1] at varying CP and amino acid formulations[2]

Primilac	CP level	Weeks of age 33 to 36[3]	37 to 40	41 to 44[4]	Total
		(eggs, no.)			
No	Adequate ad libitum[5]	24.58	25.19[ab]	22.92[a]	72.69[a]
No	Adequate	24.46	24.73[abc]	22.48[ab]	71.67[abc]
No	Marginal	23.92	23.85[c]	22.17[ab]	69.94[c]
No	Deficient	24.29	24.27[abc]	21.50[b]	70.06[bc]
Yes	Adequate ad libitum	24.71	25.33[a]	22.44[ab]	72.48[ab]
Yes	Adequate	24.83	25.02[ab]	21.90[ab]	71.75[abc]
Yes	Marginal	24.90	24.21[bc]	22.50[ab]	71.60[abc]
Yes	Deficient	24.04	24.17[bc]	21.63[b]	69.83[c]
SEM		0.24	0.25	0.27	0.57
		Probability			
Source of variation					
Primilac		0.07	0.33	0.42	0.42
CP level		0.14	0.0001	0.0005	0.0001
Primilac × CP level		0.075	0.80	0.24	0.29
Main effect means					
	Adequate ad libitum	24.65	25.26[a]	22.68[a]	72.58[a]
	Adequate	24.65	24.88[a]	22.19[a]	71.71[ab]
	Marginal	24.41	24.03[b]	22.33[a]	70.77[bc]
	Deficient	24.17	24.22[b]	21.56[b]	69.95[c]

[a–c]Means within a column with no common superscript differ as a result of Tukey means comparison ($P < 0.05$).

[1][15].

[2]Means represent 24 cages per diet, 2 birds per pen.

[3]27 d.

[4]26 d.

[5]The adequate diet was fed to 2 treatments groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day.

Primilac supplementation had no effect on the number of eggs laid (Table 6), individual egg weight (Table 7), or egg mass over the production period(s) (Table 8), despite the small reduction in daily essential AA intake (Table 4). The reduction in overall feed intake at 36 and 44 wk of age resulted in a reduction in the feed intake-to-egg mass ratio by 2.4 and 3.4%, from 33 to 36 and 41 to 44 wk, respectively (Table 9). Feed intake-to-egg mass ratio was unaffected ($P = 0.08$) by Primilac supplementation from 37 to 40 wk of age or over the duration of the study ($P = 0.16$), even though a range of improvements in feed intake-to-egg mass ratios with DFM feeding from 1.5 to 5.4% have been reported [9–12, 24].

The numbers of eggs laid was greater for birds fed the adequate CP and AA diet than for those fed the marginal or deficient diets at 37 to 40 wk and 41 to 44 wk of age. From 33 to 36 wk of age, individual egg weight was greatest for birds fed adequate AA ad libitum and was lightest for birds fed deficient AA (1.21-g difference; $P = 0.06$ for the CP level effect). Egg weight was not affected by dietary CP or AA concentration from 37 to 40 wk of age. However, from 41 to 44 wk of age, birds fed the adequate AA diet laid eggs that were at least 1.36 g heavier than those fed the marginal or deficient diets. Over the entire production period (37 to 44 wk of age), the hens fed the adequate AA diet laid 110 g more egg mass than those fed the marginal or deficient diet. Total eggs laid and egg mass were greatest when at least 14.4 g of CP, 804 mg of Lys, 382 mg of Met, 601 mg of TSAA, 502 mg of Thr, and 609 mg of Ile were consumed per hen per day from 33 to 44 wk of age.

The NRC [14] AA recommendations for laying hens are based on peer-reviewed research published between 1962 and 1989. The recom-

Table 7. Egg weight (g/egg) from hens fed diets with or without Primilac[1] at varying CP and amino acid formulations[2]

Primilac	CP level	Weeks of age 33 to 36	37 to 40	41 to 44	Average
		(g/egg)			
No	Adequate ad libitum[3]	59.30^{abc}	60.05	61.33^{abc}	60.23^{ab}
No	Adequate	60.40^{a}	60.89	61.76^{ab}	61.02^{a}
No	Marginal	59.17^{abc}	59.74	59.63^{d}	59.51^{b}
No	Deficient	58.59^{bc}	59.19	59.98^{cd}	59.25^{b}
Yes	Adequate ad libitum	60.10^{ab}	60.63	62.39^{a}	61.04^{a}
Yes	Adequate	58.60^{bc}	59.80	60.96^{abcd}	59.79^{ab}
Yes	Marginal	58.71^{bc}	60.31	60.37^{bcd}	59.78^{ab}
Yes	Deficient	58.40^{c}	59.21	60.00^{cd}	59.21^{b}
SEM		0.49	0.59	0.49	0.45
		Probability			
Source of variation					
Primilac		0.24	0.96	0.46	0.88
CP level		0.06	0.18	0.0001	0.006
Primilac × CP level		0.067	0.45	0.24	0.13
Main effect means					
	Adequate ad libitum	59.70^{a}	60.34	61.86^{a}	60.63^{a}
	Adequate	59.50^{ab}	60.35	61.36^{a}	60.40^{ab}
	Marginal	58.94^{ab}	60.02	60.00^{b}	59.64^{bc}
	Deficient	58.49^{b}	59.20	59.99^{b}	59.23^{c}

[a–d]Means within a column with no common superscript differ as a result of Tukey means comparison ($P < 0.05$).

[1][15].

[2]Means represent 24 cages per diet, 2 birds per pen.

[3]The adequate diet was fed to 2 treatment groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day.

mendation is a total AA consumption of 690, 300, 580, 470, and 650 mg/hen per day of Lys, Met, TSAA, Thr, and Ile, respectively. However, the present commercial bird is very different from commercial birds available before 1989, in part because of genetic selection and the associated effects on feed consumption [25]. Because of the experimental design of the current study, individual AA requirements of laying hens cannot be determined directly. Hence, the only AA intake that was lower than the NRC [14] recommendations was Ile for the adequate AA group at 609 mg/hen per day (6% less), but this was 3% more than recommendations from Hyline International [26]. Hyline [26] recommendations for the Hyline Variety W36 are a total AA consumption of 821, 395, 711, 525, and 593 mg/hen per day of Lys, Met, TSAA, Thr, and Ile. In the current study, the greatest response of egg mass and egg number observed was no more than 5% different than the Hyline [26] recommended intake for Lys, Met, Thr, and Ile. Intake of TSAA, however, was 15.5% less than Hyline [26] recommendations.

Bregendahl et al. [27] reported that the optimal (based on break-point analyses) AA intakes of 28- to 34-wk-old hens when egg mass was the response criterion were 585, 269, 557, 468, and 476 mg/hen per day of Lys, Met, TSAA, Thr, and Ile, respectively. A higher optimal AA intake, of 753 mg/hen per day, was obtained for Lys when the feed intake-to-egg mass ratio was the response criterion [27]. The results of the current study are in general agreement with those of Bregendahl et al. [27] for Lys intake, wherein differences in egg mass and feed utilization occurred between groups of birds that consumed 721 and 803 mg of Lys/hen per day. Notably, this value is considerably higher than the 690 mg/hen per day recommended by NRC [14].

When considering the Met and TSAA requirements of hens, differences in egg mass oc-

Table 8. Egg mass (kg/feeding period) from W36 hens fed diets with or without Primilac[1] at varying CP and amino acid formulations[2]

Primilac	CP level	Weeks of age 33 to 36[3]	37 to 40	41 to 44[4]	Total
		(kg)			
No	Adequate ad libitum[5]	1.456[abc]	1.510[ab]	1.404[a]	4.370[a]
No	Adequate	1.477[ab]	1.505[abc]	1.388[ab]	4.370[a]
No	Marginal	1.415[bc]	1.424[c]	1.321[bc]	4.159[c]
No	Deficient	1.423[abc]	1.435[bc]	1.289[c]	4.148[c]
Yes	Adequate ad libitum	1.485[a]	1.535[a]	1.400[a]	4.421[a]
Yes	Adequate	1.454[abc]	1.495[ab]	1.335[abc]	4.284[ab]
Yes	Marginal	1.461[abc]	1.458[bc]	1.358[abc]	4.275[bc]
Yes	Deficient	1.403[c]	1.429[c]	1.296[c]	4.129[c]
SEM		0.016	0.018	0.018	0.035
		Probability			
Source of variation					
Primilac		0.48	0.40	0.81	0.54
CP level		0.0009	0.0001	0.0001	0.0001
Primilac × CP level		0.07	0.50	0.085	0.03
Main effect means					
	Adequate ad libitum	1.471[a]	1.523[a]	1.402[a]	4.396[a]
	Adequate	1.466[a]	1.500[a]	1.361[ab]	4.327[a]
	Marginal	1.438[ab]	1.441[b]	1.339[b]	4.217[b]
	Deficient	1.413[b]	1.433[b]	1.293[c]	4.138[b]

[a–c]Means within a column with no common superscript differ as a result of Tukey means comparison ($P < 0.05$).

[1][15].

[2]eans represent 24 cages per diet, 2 birds per pen.

[3]7 d.

[4]6 d.

[5]The adequate diet was fed to 2 treatments groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day.

curred between groups of hens that consumed 363 to 382 and 561 to 601 mg/hen per day for Met and TSAA, respectively. These values are considerably greater than the 269 mg of Met/hen per day reported by Bregendahl et al. [27] and 300 mg of Met/hen per day by NRC [14]. Similarly, differences in egg mass occurred when Ile intake was between 540 and 609 mg/hen per day. This value is considerably (6%) lower than the 650 mg/hen per day recommended by NRC [14] and is considerably higher than the 476 mg/hen per day reported by Bregendahl et al. [27] for optimal egg mass. This brings into question whether the Ile intake in this study was adequate to maximize egg mass or production and whether it was the first-limiting AA. Notably, these differences may likely reflect the method of determining the optimal AA requirement using broken-line analyses [27]. Broken-line analyses predict the optimal amount of AA intake for a given response rather than a maximal response, as exemplified by differences in predicted requirements compared with hens fed a higher AA concentration. Difficulty arises when applying the most economical AA formulation and AA intake to commercial conditions attributable to various management and environmental variables [28].

Egg characteristics were not affected by dietary regimen (data not shown). Yolk, albumen, and shell proportions averaged 28.9 (SD ± 0.91), 61.6 (±0.99), and 9.6% (±0.67) of total egg weight, respectively. Yolk and albumen DM averaged 49.1 (±1.06) and 12.3% (±0.28), respectively.

CONCLUSIONS AND APPLICATIONS

1. Total eggs laid and egg mass were greatest when at least 14.4 g of CP, 804 mg of

Table 9. Feed intake-to-egg mass ratio from hens fed diets with or without Primilac[1] at varying CP and amino acid formulations[2]

Primilac	CP level	Weeks of age 33 to 36[3]	37 to 40	41 to 44[4]	Total
		(kg/kg)			
No	Adequate ad libitum[5]	1.555[a]	1.527[ab]	1.478[ab]	1.517[ab]
No	Adequate	1.471[bc]	1.499[b]	1.489[ab]	1.484[ab]
No	Marginal	1.496[abc]	1.562[ab]	1.455[ab]	1.505[ab]
No	Deficient	1.530[ab]	1.576[ab]	1.532[a]	1.545[a]
Yes	Adequate ad libitum	1.487[abc]	1.495[b]	1.386[b]	1.456[b]
Yes	Adequate	1.485[abc]	1.553[ab]	1.457[ab]	1.497[ab]
Yes	Marginal	1.434[c]	1.630[a]	1.426[ab]	1.494[ab]
Yes	Deficient	1.502[abc]	1.632[a]	1.484[ab]	1.538[a]
SEM		0.019	0.029	0.025	0.017
		Probability			
Source of variation					
Primilac		0.006	0.08	0.006	0.16
CP level		0.004	0.002	0.01	0.004
Primilac × CP level		0.10	0.29	0.58	0.14
Main effect means					
No		1.513	1.541	1.489	1.513
Yes		1.477	1.578	1.438	1.496
	Adequate ad libitum	1.521[a]	1.511[a]	1.432[b]	1.486[a]
	Adequate	1.478[ab]	1.526[ab]	1.473[ab]	1.491[a]
	Marginal	1.464[b]	1.596[b]	1.441[b]	1.499[ab]
	Deficient	1.516[a]	1.604[b]	1.508[a]	1.542[b]

[a–c]Means within a column with no common superscript differ as a result of Tukey means comparison ($P < 0.05$).

[1][15].

[2]Means represent 24 cages per diet, 2 birds per pen.

[3]27 d.

[4]26 d.

[5]The adequate diet was fed to 2 treatments groups of hens: 1 treatment was fed at 90 g/hen per day, and the other was fed ad libitum. The marginal and deficient diets were fed at 90 g/hen per day.

Lys, 382 mg of Met, 601 mg of TSAA, 502 mg of Thr, and 609 mg of Ile were consumed per hen per day from 33 to 44 wk of age.

2. Within the range of CP intake (12.5 to 14.5 g/hen per day), egg characteristics (shell, yolk, albumen, and solids percentages) were unaffected.
3. Contrary to our hypothesis, supplementation with the DFM (Primilac) was not able to alleviate a marginal AA deficiency in laying hens; however, it did improve feed intake-to-egg mass ratios during 8 wk of the 12-wk study.

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