Requirement of Tryptophan in Relation to the Supply of Large Neutral Amino Acids in Laying Hens

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ABSTRACT The present study was undertaken to find out whether the tryptophan requirement of laying hens is influenced by the supply of large neutral amino acids (LNAA). A factorial experiment was performed in which the dietary tryptophan concentration was varied at six different levels (1.0, 1.25, 1.5, 1.75, 2.0, and 2.5 g tryptophan/kg diet). As the second factor, the dietary concentrations of LNAA (isoleucine, valine, leucine, phenylalanine, and tyrosine) were varied at two levels. The first level provided an adequate supply of these amino acids; at the second level the concentrations of these amino acids were 40% higher than at the first level. The tryptophan requirement was estimated by a broken-line model and an exponential model of regression analysis. The tryptophan intake required for optimum (100% of maximum in the broken-line model, 95% of the maximum in the exponential model) egg production and daily egg mass was lower in hens fed the diets with high LNAA concentrations (145 and 155 mg/hen per day, respectively, in average of both models) than in hens fed the diets with adequate concentrations of LNAA (184 and 198 mg/hen per day, respectively, in average of both models). In contrast, the tryptophan requirement for optimum BW gain was lower in hens fed the diets with adequate LNAA concentrations (178 mg tryptophan per day) than in hens fed the diets with a high concentration of LNAA (212 mg tryptophan per day). In conclusion, the study suggests that an interaction between dietary LNAA and tryptophan exists in laying hens.

(*Key words*: laying hen, tryptophan, large neutral amino acid, egg mass, body weight change)

2003 Poultry Science 82:815–822

INTRODUCTION

Tryptophan plays a significant role in the use of crude protein-reduced rations in laying hen nutrition because it is considered to be the third-limiting amino acid, after the sulfur-containing amino acids and lysine. The supply of sulfur-containing amino acids and lysine is generally assured in modern poultry nutrition, even with protein-reduced rations, because these amino acids can be supplemented in their free form. In the case of tryptophan this form of supplementation is currently not economically viable, which is why an adequate amount has to be provided with the diet. However, the existing database regarding the tryptophan requirement for laying hens is inconsistent. Several researchers have reported that a tryptophan intake of 117 to 239 mg/hen per d or a dietary tryptophan concentration between 1.1 and 1.9 g tryptophan/kg diet is necessary to optimize or maximize performance (Bray, 1969; Wethli and Morris, 1978; Ishibashi, 1985; Othani et al., 1989; Jensen et al., 1990; Harms and Russell, 2000). It should be noted that the hen's age, daily egg mass, and composition of the rations in these experiments varied.

Studies with pigs have shown that the requirement for tryptophan is affected by the supply of large neutral amino acids (LNAA) isoleucine, valine, leucine, phenylalanine, tyrosine, methionine, and histidine and, hence, is indirectly affected by protein intake (Boomgaardt and Baker, 1973; Henry et al., 1992; Peisker et al., 1998). The interactions result from the competition of these amino acids for transfer into the brain. These phenomena have so far not been studied in laying hens but can be postulated. The present study therefore aims to investigate whether the tryptophan requirement of laying hens is dependent on the supply of LNAA. A factorial experiment was set up in which the dietary concentrations of tryptophan and LNAA were varied. The amount of tryptophan required to optimize various performance parameters at different dietary levels of LNAA was determined by means of regression analysis.

MATERIALS AND METHODS

An experiment was conducted with 144 Lohmann Brown layers from 31 to 37 wk of age. The experimental

Received for publication July 30, 2002.
Accepted for publication January 15, 2003.

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Abbreviation Key: LNAA = large neutral amino acids.

TABLE 1. Composition of the basal experimental diet

Ingredient	Amount (g/kg)
Corn	328
Wheat	170
Peas	160
Barley	150
Corn gluten feed	43.6
Soybean oil	20
Calcium carbonate	86
Calcium phosphate	14
Vitamin and mineral premix[1]	10
Salt	1.3
L-Lysine-HCl	1.8
DL-Methionine	1.7
L-Threonine	0.5
L-Isoleucine	1.6
L-Valine	1.0
L-Aspartic acid	4.7
L-Glutamic acid	7.5
Analysis (g/kg)	
Crude protein	125
Crude fat	42
Crude fiber	44
Crude ash	130
Methionine	3.5
Methionine + cysteine	6.0
Lysine	6.2
Tryptophan	1.0
Threonine	4.5
Isoleucine	5.7
Valine	6.3
Leucine	9.8
Phenylalanine	5.4
Tyrosine	3.8
Calcium	36.2
Total phosphorus	6.1
Nonphytic acid phosphorus	4.2
Energy (MJ ME/kg), calculated[2]	11.45

[1]Supplied per kilogram of diet: calcium, 1.70 g; sodium, 0.80 g; vitamin A, 12,000 IU; cholecalciferol, 2,500 IU; DL-α-tocopherol acetate, 20 mg; thiamine, 5 mg; riboflavine, 3 mg; pyridoxine, 3 mg; vitamin B_{12}, 20 μg; vitamin K_3, 1.2 mg; pantothenic acid, 8 mg; niacin, 30 mg; folic acid, 0.5 mg; choline chloride 150 mg; iron, 25 mg; zinc, 60 mg; manganese, 100 mg; copper, 5 mg; cobalt, 0.1 mg; iodine, 1 mg; selenium, 0.2 mg.

[2]Calculated according to data provided by Jahrbuch für die Geflügelwirtschaft (2000).

design was factorial and involved varying the dietary concentrations of tryptophan and LNAA. A tryptophan-deficient and protein-reduced basal diet was used, which consisted primarily of cereal and peas and contained 11.4 MJ ME/kg (Table 1). The dietary tryptophan concentrations were varied at six levels (1.0, 1.25, 1.5, 1.75, 2.0, and 2.5 g tryptophan/kg diet) by adding L-tryptophan of at least purity 98%[2] to the basal diet. The dietary concentrations of LNAA (isoleucine, valine, leucine, phenylalanine, and tyrosine) were varied by supplementing the respective amino acids in their free form (supplied by Lohmann Animal Health, purity at least 98%) at two levels. The first level was based on the recommendations of the German Nutrition Society (1999) for these amino acids (isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet). The second level of concentrations of these amino acids were 40% higher than in the first level (isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet). The concentrations of other essential amino acids were adjusted to an adequate level as recommended by the German Nutrition Society (1999) by supplementation with synthetic amino acids (see Table 1).

Twelve treatment groups of 12 hens each were used. The hens were kept one bird per cage in an environmentally controlled room at 18°C. The room was lit for 14 h daily at 20 to 30 lx. Feed in mash form and water (via nipple drinkers) were available ad libitum. The experiment was conducted over a 6-wk period. All procedures followed established guidelines for the care and handling of animals and were approved by the veterinary council of Saxony-Anhalt.

The following data were recorded: BW at the start and end of the experiment, weekly feed consumption, and number of eggs daily. Egg weight was determined from two eggs from each hen at the end of each week. Additionally, on the last day of the experiment a blood sample was drawn from the jugular vein of each hen (after fasting for 7 h) of the groups receiving the diets with tryptophan concentrations of 1.0, 1.5, 1.75, and 2.5 g tryptophan/kg diet to measure the concentrations of free amino acids in the plasma. Plasma amino acids of the groups receiving the diets with 1.25 and 2.0 g tryptophan/kg diet were not analyzed because the number of amino acid analyses was limited for technical reasons.

The crude nutrient concentrations of the diets were analyzed according to official VDLUFA methods (Naumann and Bassler, 1976, 1988, 1993). Concentrations of amino acids were determined by hydrolyzing the diets with 6 *N* hydrochloric acid; the pH of the hydrolysate was adjusted to pH 2.2. Amino acids were separated and quantified by ion exchange chromatography in an amino acid analyzer.[3] A special separating column with cation exchanger resin[4] was used for this purpose. Elution was performed with a gradient system consisting of four buffers of pH 3.40, 3.60, 4.50, and 11.0 and a standard buffer of pH 2.20. Detection took place following post-column derivatization at a wavelength of 570 nm. The tryptophan concentration of the diets was determined by reverse-phase HPLC. Separation was done on an RP-18-e column (5 μm particle size, 250 × 4 mm);[3] elution was with a gradient of potassium dihydrogenphosphate (0.01 *M*, with 8% methanol) and methanol. Detection was by fluorescence at 280 nm (excitation) and 355 nm (emission).

The free amino acids in the blood plasma were also determined by reverse-phase HPLC. The separation of the indole derivatives was performed following precolumn derivatization with ortho-phthaldialdehyde and mercaptopropionic acid. Derivatization was done using the method of Teerlink et al. (1994). Chromatography

[2]Lohmann Animal Health, Cuxhaven, Germany.
[3]Biotronik LC3000, Eppendorf, Hamburg, Germany.
[4]Laborservice Onken, Gruendau, Germany.

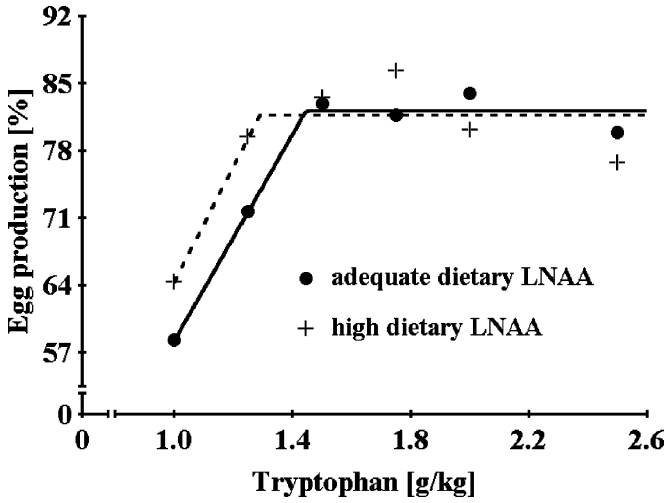

FIGURE 1. Regression analysis of the egg production as a function of the dietary tryptophan concentration by the broken-line model for hens receiving diets with adequate large neutral amino acids (LNAA) concentration (——; $y_1 = 4.7 + 54 \cdot x$, for $x \leq c$; $y_2 = 4.7 + 54 \cdot 1.44$, for $x > c$; y = egg production, %; x = tryptophan concentration, g/kg diet) or high LNAA concentrations (– – – –; $y_1 = 4.4 + 60 \cdot x$, for $x \leq c$; $y_2 = 4.4 + 60 \cdot 1.29$, for $x > c$; y = egg production, %; x = tryptophan concentration, g/kg diet). Diets with adequate LNAA concentrations contained isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet. Diets with high LNAA concentrations contained isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet.

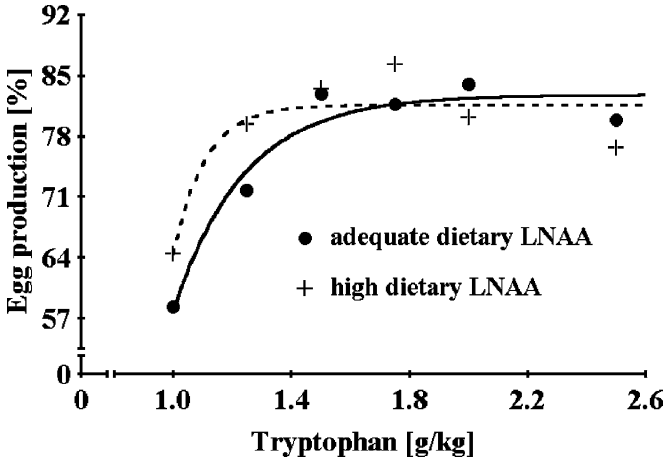

FIGURE 2. Regression analysis of the egg production as a function of the dietary tryptophan concentration by the exponential model for hens receiving diets with adequate large neutral amino acids (LNAA) concentration (——; $y = 57.9 + 24.9 \cdot (1 - e^{-4.2\,(x\,-\,1.0)})$; y = egg production, %, x = tryptophan concentration, g/kg diet) or high LNAA concentrations (– – – –; $y = 64.4 + 17.2 \cdot (1 - e^{-9.3\,(x\,-\,1.0)})$; y = egg production, %, x = tryptophan concentration, g/kg diet). Diets with adequate LNAA concentrations contained isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet. Diets with high LNAA concentrations contained isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet.

was based on the method of Schuster (1988). Amino acid derivatives were separated on a Hypersil ODS column (5 μm, 250 × 4 mm)[5] using an HPLC apparatus.[6] Detection was by fluorescence detector at an excitation wavelength of 337 nm and an emission wavelength of 454 nm based on the method of Molnar-Perl and Vasanits (1999).

The statistical analysis of the data was performed with the software package Statistica for Windows (StatSoft, Inc., 2000). The data were tested for normal distribution and homogeneity of the variances. Data were evaluated by ANOVA with main the factors being dietary tryptophan, LNAA concentrations, and the interaction between them. Performance parameters were subjected to separate analyses of variance for dietary tryptophan concentrations in the range of 1.0 to 1.25 g/kg diet and dietary tryptophan concentrations in the range of 1.5 to 2.5 g/kg diet in order to compare the effects of dietary LNAA at insufficient and adequate dietary tryptophan concentrations. When *F*-values were significant ($P < 0.05$), the means were compared by the Newman-Keuls test. To assess the tryptophan requirement of hens fed diets with adequate or high LNAA concentrations, the data of feed consumption, egg mass, egg production, and BW change were subjected to regression analysis by the broken-line model (Robbins et al., 1979) and an exponential model. The equation of the broken-line model was:

$$y = a + b \cdot x, \text{ for } x \leq c \text{ and}$$
$$y = a + b \cdot c, \text{ for } x > c,$$

where y = response criteria, a = ordinate of the breakpoint, b = slope of the line for $x \leq c$, c = abscissa of the breakpoint (= requirement), and x = tryptophan concentration of the diet. In the broken-line model, the dietary tryptophan concentration was calculated that was required to achieve the maximum of the performance parameter considered. The equation of the exponential model was

$$y = a + b \cdot (1 - e^{-c(x-d)}),$$

where y = response criteria, a = intercept (performance of the basal diet), b = maximum response due to increased tryptophan concentration, c = slope, d = tryptophan concentration of the basal diet, and x = tryptophan concentration of the diet. In the exponential model, the dietary tryptophan concentration was calculated that was required to achieve 95% of the maximum of the performance parameter considered.

RESULTS

The dietary tryptophan concentration had a significant effect on all studied performance characteristics (Table 2). At both dietary LNAA concentrations, feed consumption, egg production, and BW change of the

[5]Hewlett Packard, Waldbronn, Germany.
[6]1100 series, Agilent Technologies, Waldbronn, Germany.

TABLE 2. Effect of the dietary tryptophan concentration on performance of laying hens receiving diets with adequate or high dietary concentrations of large neutral amino acids (LNAA) from 31 to 37 wk of age[1]

Dietary tryptophan (g/kg diet)	Feed consumption (g/hen/d)	Egg production (%)	Egg mass (g/hen/d)	Body weight change (g)	Trp intake (mg/hen/d)
	— Adequate dietary LNAA concentration[2] —				
1.00	87.0[b]	58.3[b]	34.0[c]	–223[b]	87[e]
1.25	105.0[a]	71.7[a]	41.2[b]	–73[ab]	131[d]
1.50	117.9[a]	82.9[a]	50.0[a]	+11[a]	177[c]
1.75	113.0[a]	81.7[a]	48.2[a]	+42[a]	198[c]
2.00	120.6[a]	84.0[a]	50.3[a]	+42[a]	241[b]
2.50	111.4[a]	79.9[a]	48.3[a]	–14[a]	279[a]
Pooled SEM	5.1	3.3	1.4	61	10
	— High dietary LNAA concentration[3] —				
1.00	86.5[b]	64.4[b]	36.5[b]	–265[b]	86[e]
1.25	108.2[a]	79.4[a]	46.3[a]	–100[a]	135[d]
1.50	110.9[a]	83.5[a]	49.6[a]	–59[a]	166[c]
1.75	121.9[a]	86.3[a]	51.5[a]	+43[a]	213[b]
2.00	113.0[a]	80.2[a]	47.3[a]	+17[a]	226[b]
2.50	110.3[a]	76.7[a]	47.2[a]	–42[a]	276[a]
Pooled SEM	5.0	4.1	1.7	57	9
	— Results of ANOVA (*P*-Value) —				
Trp = 1.0 – 1.25 g/kg diet					
Trp	0.001	0.001	0.001	0.02	0.001
LNAA	0.81	0.08	0.02	0.58	0.79
Trp × LNAA	0.74	0.83	0.39	0.91	0.73
Trp = 1.50 – 2.50 g/kg diet					
Trp	0.51	0.42	0.49	0.50	0.001
LNAA	0.62	0.87	0.77	0.46	0.79
Trp × LNAA	0.29	0.65	0.25	0.94	0.73

[a–e]Means with the same superscript within a column do not differ significantly ($P < 0.05$).

[1]Results are means with n = 12 per treatment.

[2]Isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet.

[3]Isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet.

hens showed their maximum at tryptophan concentrations of 1.25 to 2.50 g/kg diet but were significantly lower at 1.0 g of tryptophan/kg diet. The effect of dietary tryptophan concentration on daily egg mass depended on dietary LNAA concentration. At the adequate dietary LNAA concentration, maximum daily egg mass was observed at dietary tryptophan concentrations of 1.50 to 2.50 g/kg diet; at the high dietary LNAA concentration, maximum daily egg mass was reached even at a lower dietary tryptophan concentration of 1.25 g/kg diet.

The ANOVA, which only covered the low dietary tryptophan concentrations of 1.0 and 1.25 g/kg diet, also revealed effects of the dietary LNAA concentration on daily egg mass. At low dietary tryptophan concentrations of 1.0 and 1.25 g/kg diet, daily egg mass was higher ($P < 0.05$) in hens fed diets with high LNAA concentrations than in hens fed diets with the adequate LNAA concentrations. At the high dietary LNAA concentration, there was also a tendency ($P < 0.10$) toward a higher egg production than at the adequate LNAA concentration. At dietary tryptophan concentrations between 1.5 and 2.5 g/kg diet, the concentration of dietary LNAA did not influence any of the performance characteristics.

According to the broken-line model and the exponential model, the dietary tryptophan intake to reach the optimum (100% of maximum in the broken-line-model; 95% of maximum in the exponential model) of egg production and egg mass was higher in hens fed diets with adequate dietary LNAA concentrations than in hens fed diets with high LNAA concentrations (Table 3, Figures 1, 2, 3, 4). At adequate dietary LNAA, optimum egg production and egg mass were reached at dietary tryptophan intakes of 184 and 198 mg/hen per day, respectively, in average of both models; at the high dietary LNAA concentration, optimum of these parameters was reached at dietary tryptophan intakes of 145 and 155 mg/hen per d, respectively, in average of both models. The situation with regard to BW change was the reverse of that for egg production and daily egg mass. At adequate dietary LNAA concentration, hens required 178 mg tryptophan/hen per day, in average of both models to reach optimum of BW gain; at the high dietary LNAA concentration, hens required 212 mg tryptophan/hen per day to reach optimum body weight gain. The amount of tryptophan required to achieve the optimum of feed consumption was similar in hens fed diets with adequate LNAA concentrations (171 mg/hen per day, in average of both models) and those fed diets with high

TABLE 3. Tryptophan requirement for optimum feed consumption, egg production, egg mass, and body weight change in laying hens receiving diets with adequate or high dietary concentrations of large neutral amino acids (LNAA) assessed by the broken-line model and the exponential model

	Broken-Line		Exponential	
Dietary LNAA concentration	Adequate[2]	High[3]	Adequate[2]	High[3]
Feed consumption				
Optimum[1] (g/hen/d)	115.8	114.0	110.3	108.7
Tryptophan concentration (g/kg diet)	1.40	1.32	1.62	1.49
Tryptophan intake (mg/hen/d)	162	151	179	162
Statistical range (g/kg)	1.29–1.51	1.23–1.41	1.40–2.33	1.30–2.41
Fit (R^2)	0.93	0.87	0.90	0.89
Egg production				
Optimum[1] (%)	81.9	81.8	81.5	80.7
Tryptophan concentration (g/kg diet)	1.44	1.29	1.71	1.32
Tryptophan intake (mg/hen/d)	171	144	197	146
Statistical range (g/kg)	1.34–1.54	1.19–1.39	1.54–2.04	1.16–2.26
Fit (R^2)	0.98	0.82	0.95	0.82
Egg mass				
Optimum[1] (g/hen/d)	49.2	49.0	49.0	48.3
Tryptophan concentration (g/kg diet)	1.50	1.32	1.82	1.42
Tryptophan intake (mg/hen/d)	185	151	210	159
Statistical range (g/kg)	1.46–1.54	1.25–1.39	1.60–2.30	1.27–1.95
Fit (R^2)	0.98	0.91	0.93	0.90
Body weight change				
Optimum[1] (g)	23.0	7.0	13.9	10.8
Tryptophan concentration (g/kg diet)	1.41	1.61	1.67	1.74
Tryptophan intake (mg/hen/d)	164	225	192	199
Statistical range (g/kg)	1.31–1.51	1.46–1.76	1.50–2.00	1.52–2.33
Fit (R^2)	0.96	0.90	0.94	0.90

[1]In the broken-line-model, the optimum refers to 100% of the maximum of the parameter considered; in the exponential model, optimum refers to 95% of the maximum of the parameter considered.

[2]Isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet.

[3]Isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet.

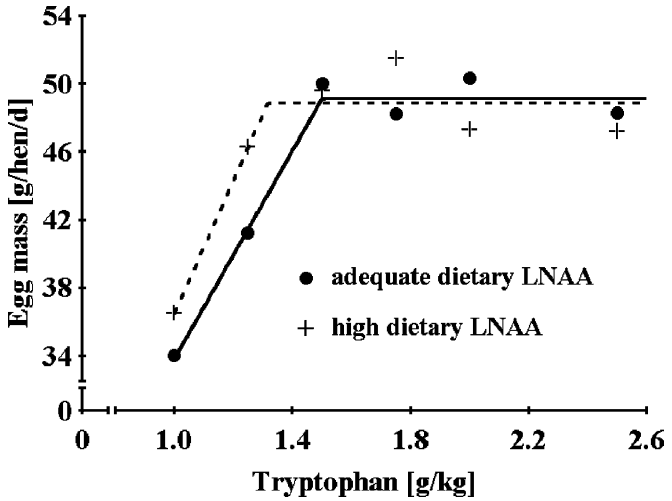

FIGURE 3. Regression analysis of the egg mass as a function of the dietary tryptophan concentration by the broken-line model for hens receiving diets with adequate large neutral amino acids (LNAA) concentration (——; $y_1 = 3.2 + 31 \cdot x$, for $x \le c$; $y_2 = 3.2 + 31 \cdot 1.50$, for $x > c$; y = egg mass, g/hen/d; x = tryptophan concentration, g/kg diet) or high LNAA concentrations (– – – –; $y_1 = -2.7 + 39 \cdot x$, for $x \le c$; $y_2 = -2.7 + 39 \cdot 1.32$, for $x > c$; y = egg mass, g/hen/d; x = tryptophan concentration, g/kg diet). Diets with adequate LNAA concentrations contained isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet. Diets with high LNAA concentrations contained isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet.

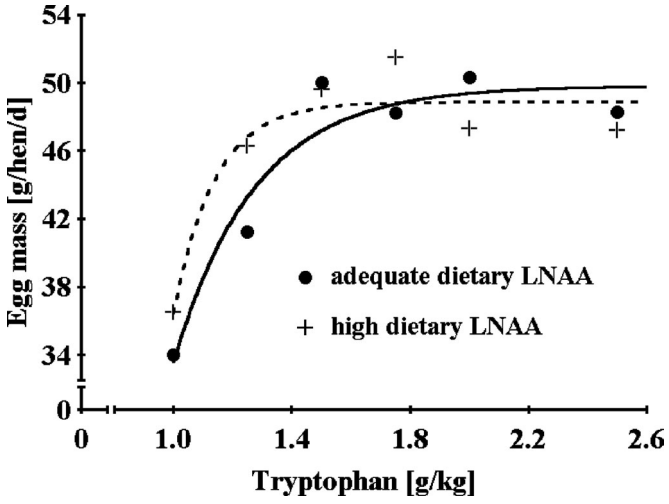

FIGURE 4. Regression analysis of the egg mass as a function of the dietary tryptophan concentration by the exponential model for hens receiving diets with adequate large neutral amino acids (LNAA) concentration (——; $y = 33.6 + 16.2 \cdot (1 - e^{-3.7\,(x\,-\,1.0)})$; y = egg mass, g/hen/d, x = tryptophan concentration, g/kg diet) or high LNAA concentrations (– – – –; $y = 36.5 + 12.4 \cdot (1 - e^{-7.2\,(x\,-\,1.0)})$; y = egg mass, g/hen/d, x = tryptophan concentration, g/kg diet). Diets with adequate LNAA concentrations contained isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet. Diets with high LNAA concentrations contained isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet.

TABLE 4. Effect of the dietary tryptophan concentration on concentrations of free tryptophan and large neutral amino acids (LNAA) and their ratio in plasma of laying hens receiving diets with adequate or high dietary LNAA concentrations from 31 to 37 wk of age[1]

Dietary tryptophan (g/kg diet)	Trp (μmol/L)	Σ LNAA[2] (μmol/L)	Trp/Σ LNAA ($\times$ 100)
	Adequate dietary LNAA concentration[3]		
1.00	14[b]	449	3.1[b]
1.50	33[a]	474	7.1[a]
1.75	35[a]	495	7.2[a]
2.50	38[a]	499	7.5[a]
Pooled SEM	2	18	0.4
	High dietary LNAA concentration[4]		
1.00	13[c]	500[b]	2.8[b]
1.50	33[b]	546[a]	6.1[a]
1.75	37[ab]	554[a]	6.8[a]
2.50	40[a]	567[a]	7.2[a]
Pooled SEM	2	18	0.3
	Results of ANOVA (P-Value)		
Trp = 1.0 – 2.50 g/kg diet			
Trp	0.001	0.001	0.001
LNAA	0.49	0.001	0.06
Trp × LNAA	0.78	0.87	0.83

[a–c]Means with the same superscript within a column do not differ significantly ($P < 0.05$).

[1]Results are means with n = 12 per treatment.

[2]Sum of isoleucine, valine, leucine, phenylalanine, tyrosine.

[3]Isoleucine, 5.7 g/kg diet; valine, 6.3 g/kg diet; leucine, 9.8 g/kg diet; phenylalanine, 5.4 g/kg diet; tyrosine, 3.8 g/kg diet.

[4]Isoleucine, 8.0 g/kg diet; valine, 8.8 g/kg diet; leucine, 13.7 g/kg diet; phenylalanine, 7.6 g/kg diet; tyrosine, 5.3 g/kg diet.

LNAA concentrations (156 mg/hen per day, in average of both models).

Increasing the dietary tryptophan concentrations above 1.0 g/kg diet caused a significant rise in the tryptophan concentration and in the ratio of tryptophan to LNAA in the blood plasma (Table 4). At adequate dietary LNAA concentration, plasma tryptophan concentrations were maximum at dietary tryptophan concentrations between 1.50 and 2.50 g/kg diet; at the high dietary LNAA concentration, plasma tryptophan concentrations reached maximum at dietary tryptophan concentrations of 1.75 or 2.50 g/kg diet. The concentrations of LNAA in plasma were, according to ANOVA, significantly higher in hens fed the diets with high LNAA concentrations than in the hens fed the diets with adequate LNAA concentrations. The concentration of LNAA in plasma was also influenced by dietary tryptophan concentration. According to factorial ANOVA, it was significantly higher in hens fed diets with tryptophan concentrations between 1.50 and 2.50 g/kg diet than in hens fed diets containing 1.0 g/kg diet. The ratio of tryptophan to LNAA plasma was influenced by dietary tryptophan concentration. At both dietary LNAA concentrations, it was highest in hens fed diets with tryptophan concentrations between 1.50 and 2.50 g/kg diet; in hens fed diets with 1.0 g tryptophan/kg diet, it was significantly lower. The dietary LNAA concentration did not significantly influence the ratio between tryptophan and LNAA in plasma.

DISCUSSION

The study reported here was performed to find out whether the concentration of dietary LNAA influences the requirement of tryptophan in laying hens for optimum laying performance. To assess the tryptophan requirement from the performance data, two regression models were used, the broken-line model and the exponential model. The broken-line model tends to underestimate the requirement, whereas the exponential model tends to overestimate the requirement (Fisher et al., 1973; Fuller and Garthwaite, 1993). Accordingly, the daily tryptophan intakes required for optimum egg mass, egg production, feed consumption, and BW calculated by the exponential model were higher than those calculated by the broken-line model for maximum of those parameters. The average of both models might provide the most accurate value and was therefore used in this study to derive the tryptophan requirement of hens. The results of regression analysis showed that hens receiving a diet with high concentrations of LNAA required less tryptophan to achieve optimum of egg production and daily egg mass than hens receiving a diet with adequate LNAA concentrations. However, hens receiving diets with a high LNAA concentration lost a significant amount of BW at the tryptophan level needed for optimum egg production and daily egg mass, whereas hens fed the diets with adequate LNAA concentrations did not lose BW. This finding suggests that supplementation of LNAA combined with a low trypto-

phan intake enhances the mobilization of tryptophan from body pools, which could be utilized for the formation of egg mass. The observation that hens fed the diets with high LNAA concentrations required more tryptophan for achieving optimum BW gain than hens fed the diets with adequate LNAA concentrations also suggests that an excess of LNAA either promotes the mobilization of tryptophan from body pools or inhibits its incorporation into the body.

Increased mobilization of tryptophan from body pools by high dietary LNAA concentrations could also explain the finding that supplementation of LNAA increased daily egg mass at low dietary tryptophan concentrations of 1.0 or 1.25 g/kg diet. We assume that supplementation of LNAA over the requirement of the hens modified the relative distribution of tryptophan between egg protein and body protein. Further research is, however, needed to confirm this assumption and to determine mechanisms by which the LNAA influences the metabolism of tryptophan and its utilization for egg production in laying hens. To date, no other studies exist that have investigated metabolic interactions between dietary LNAA and tryptophan in poultry.

Several studies with various species have shown that a low tryptophan intake associated with low plasma tryptophan concentrations causes anorexia rats (Bleiberg-Daniel et al., 1990) and pigs (Seve et al., 1991). This effect is due to the role of tryptophan as a precursor of serotonin, which is an important regulator of the appetite of man and animals (Blundell and Latham, 1978; Tackman et al., 1990; Roca et al., 1999). In pigs, it has been shown that high dietary LNAA concentrations reduce feed consumption due to suppression of the transfer of tryptophan into the brain across the blood-brain barrier and sequent formation of serotonin in the brain (Henry et al., 1992). Laying hens also responded to low dietary tryptophan concentrations with markedly lower feed consumption, as was expected. However, concentrations of tryptophan in plasma and the ratio between tryptophan and LNAA in the plasma were similar at both dietary LNAA concentrations. It was therefore not surprising that feed consumption was independent of dietary LNAA concentration.

The tryptophan requirement for optimum daily egg mass (198 mg tryptophan/d for a daily egg mass of 49 g, in average of the models used) at the adequate dietary LNAA concentration found in the present study was distinctly higher than the suggestions by other authors (Bray, 1969, 117 mg for a daily egg mass of 46 g; Morris and Wethli, 1978, 182 mg for a daily egg mass of 50 g; Jensen et al., 1990, 124 to 168 mg for a daily egg mass of 46 to 52 g; Schutte, 1998, 180 mg for a daily egg mass of 55 g; Russell and Harms, 1999, 157 mg for a daily egg mass of 55 g; Harms und Russell, 2000, 149 mg tryptophan per day for a daily egg mass of 50 g) and also higher than the recommendations of the NRC (1994; 175 mg tryptophan per day for brown-egg layers with an egg production of 90%). The results of the present study also imply that the requirement of laying hens for tryptophan is presently slightly underestimated.

ACKNOWLEDGMENT

Financial support of Lohmann Animal Health (Cuxhaven, Germany) is gratefully acknowledged.

REFERENCES

Bleiberg-Daniel, F., B. Le Moullac, J. C. Maire, and S. Wade. 1990. Failure of tryptophan deficiency to reduce specifically serum levels of transthyretin or albumin in rats. J. Nutr. 120:1610–1616.

Blundell, J. E., and C. J. Latham. 1978. Pharmacological manipulation of feeding behavior: Possible influences of serotonin and dopamine on food intake. Pages 83–109 in Central Mechanism of Anorectic Drugs. S. Garattini and R. Samanin, ed. Raven Press, New York.

Boomgaardt, J., and D. H. Baker. 1973. Tryptophan requirement of growing pigs at three levels of dietary protein. J. Anim. Sci. 36:303–306.

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

Fisher, C., T. R. Morris, and J. R. Jennings. 1973. A model for the description and prediction of the response of laying hens to amino acid intake. Br. Poult. Sci. 14:469–484.

Fuller, M. F., and P. Garthwaite. 1993. The form of response of body protein accretion to dietary amino acid supply. J. Nutr. 123:957–963.

German Nutrition Society (GfE). 1999. Energie- und Nährstoffbedarf Landwirtschaftlicher Nutztiere, Nr.7. Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler). Verlag, Frankfurt.

Harms, R. H., and G. B. Russell. 2000. Evaluation on tryptophan requirement of the commercial layer by using a corn-soybean meal basal diet. Poult. Sci. 79:740–742.

Henry, Y., B. Seve, Y. Colleaux, P. Ganier, C. Saligaut, and P. Jego. 1992. Interactive effects of dietary levels of tryptophan and protein on feed intake and growth performance in pigs, in relation to plasma free amino acids and hypothalamic serotonin. J. Anim. Sci. 70:1873–1887.

Ishibashi, T. 1985. Tryptophan requirement of laying hens. Jpn. Poult. Sci. 22:256–263.

Jahrbuch für die Geflügelwirtschaft. 2000. Nährstoff-, Mineralstoff- und Aminosäurentabelle zur Geflügelfütterung. Verlag Eugen Ulmer, Stuttgart.

Jensen, L. S., V. M. Calderon, and C. X. Mendonca. 1990. Response to tryptophan of laying hens fed practical diets varying in protein concentration. Poult. Sci. 69:1956–1965.

Molnar-Perl, I., and A. Vasanits. 1999. Stability and characteristics of the *o*-phthaldialdehyde/*ss*-mercaptopropionic acid and *o*-phthaldialdehyde/*N*-acetyl-L-cysteine reagents and their amino acids derivatives measured by high-performance liquid chromatography. J. Chromatogr. 835:73–91.

Morris, T. R., and E. Wethli. 1978. The tryptophan requirements of young laying pullets. Br. Poult. Sci. 19:455–466.

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

Naumann, C., and R. Bassler. 1976. Methodenbuch Band III. Die chemische Untersuchung von Futtermitteln, mit 2. Ergänzungslieferung (1988) und 3. Ergänzungslieferung (1993). VDLUFA-Verlag, Darmstadt, Germany.

Othani, H., S. Saitoh, and H. Ohkawara. 1989. Research Note: Production performance of laying hens fed L-tryptophan. Poult. Sci. 68:323–326.

Peisker, M., P. H. Simmins, H. Monge, and F. Liebert. 1998. Tryptophan as a feed intake stimulant. Feed Mix 6:8–12.
Robbins, K. R., H. W. Norton, and D. H. Baker. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710–1714.
Roca, P., A. M. Proenza, and A. Palou. 1999. Sex differences in the effect of obesity on human plasma tryptophan/large neutral amino acid ratio. Ann. Nutr. Metab. 43:145–151.
Russell, G. B., and R. H. Harms. 1999. Tryptophan requirement of the commercial hen. Poult. Sci. 78:1283–1285.
Schuster, R. 1988. Determination of amino acids in biological, pharmaceutical, plant and food samples by automated precolumn derivatization and high-performance liquid chromatography. J. Chromatogr. 431:271–284.
Schutte, J. B. 1998. The ideal amino acid profile for laying hens and broiler chicks. Page 13 in Proceedings Arkansas Nutrition Conference, Fayetteville, AR.
Seve, B., M. C. Meunier-Salaün, M. Monnier, Y. Colleaux, and Y. Henry. 1991. Impact of dietary tryptophan and behavioral type on growth performance and plasma amino acids of young pigs. J. Anim. Sci. 69:3679–3688.
StatSoft, Inc. 2000. STATISTICA for Windows, Version 5.5. StatSoft. Inc., Tulsa, OK.
Tackman, J. M., J. K. Tews, and A. E. Harper. 1990. Dietary disproportions of amino acids in the rat: effects on food intake, plasma and brain amino acids and brain serotonin. J. Nutr. 120:521–533.
Teerlink, T., P. A. M. van Leeuwen, and A. Houdijk. 1994. Plasma amino acids determined by liquid chromatography within 17 minutes. Clin. Chem. 40:245–249.
Wethli, E., and T. R. Morris. 1978. Effects of age on the tryptophan requirement of laying hens. Br. Poult. Sci. 19:559–565.