Tryptophan Requirement of Laying Hens

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The requirement for each of the essential amino acids in laying hens is now systematically investigating beginning with tryptophan. Tryptophan is sometimes the first limiting amino acid of poultry rations to which methionine and lysine are added. Though a tryptophan requirement of 0.14% was recommended by the NRC¹⁾, recently TASAKI²⁾ and MORRIS and WETHLI³⁾ recommended 0.17% of tryptophan as a better requirement for laying hens, and in some cases it might be of advantage to raise the tryptophan content in the diet to more than 0.2%. These figures were estimated by use of egg production rate as the criterion. Though egg production is a very sensitive measure for the determination of amino acid requirements of laying hens, it requires long-term feeding experiments to establish valid requirement levels.

ZIMMERMAN and SCOTT⁴⁾ measured the concentration of amino acids in plasma when chicks were given an otherwise adequate diet containing graded levels of arginine, lysine or valine and concluded that the response curve could be used as a basis for determining the amino acid requirements of the chick. Amino acid requirements determined from the plasma amino acid response curve were in close agreement with values suggested using growth rate and nitrogen balance as criteria in other species⁵⁻⁷⁾. In 1976, CHI and SPEERS⁸⁾ reported that the response of plasma lysine to the dietary lysine adequacy was similarly sensitive as compared with those of egg production and nitrogen retention, suggesting that plasma amino acid assay can be used to determine the requirement of laying hens for lysine and perhaps other amino acids. If the plasma amino acid assays could be conducted in a shorter feeding period than egg production experiments, it might be possible to reduce the feeding time and expenses for evaluation of amino acid requirements of laying hens. In this paper, the tryptophan requirement was estimated by egg production rate and plasma amino acid assay.

Materials and methods

Animals and diets. Twenty-five 60-month-old Single Comb White Leghorn hens of egg production higher than 75% were selected from a large flock of hens and allotted to 5 groups of 5 birds each. The hens were housed in single cages in a room with controlled 14 hrs light (5:00-19:00) and fed diet and water *ad libitum*. The weight of eggs produced and feed consumption were recorded at 15:00 every day. Body weight was checked before and after the experiment.

The experimental diets were designed to provide the same amount of essential amino acids except for tryptophan as found in whole egg protein. All diets contained crude protein equivalent to 15.0% by adding an amino acid mixture (Table 1). The same amounts

Table 1 Composition of diet (g/kg of diet)

	I	II-III	IV
Corn with 2% fish meal	750	750	750
Alfalfa meal dehydrated	50	50	50
Calcium carbonate (shell)	60	60	60
Dicalcium phosphate	20	20	20
Mineral and vitamin mixture ¹⁾	6	6	6
Corn oil	30	26	30
Whole egg powder	—	35	10
Sodium bicarbonate	1	1	3
Agar	3	2	3
Amino acid mixture ²⁾	76.3	32.9	65.7
Tryptophan	0.93-3.20	0.86-1.71	1.12-2.30
Crude protein (analysis %)	15.1	15.4	15.2
ME (calculation kcal/kg)	3050	2990	3020

1) 6 g of mixture contained the same amounts of minerals and vitamins as recommended by the NRC¹⁾. Total niacin contents were not less than 38 mg/kg.

2) Each amino acid was added to give a total amino acid level in the diet similar to that in 150 g whole egg protein as follows: Arg, 8.56; Lys, 8.41; Met, 4.65; Cys, 3.90; Gly, 5.25; Ile, 8.10; Leu, 1.290; Phe, 7.80; Tyr, 6.00; Thr, 7.05; Val, 9.90; His, 2.68; Pro, 6.75 mg/kg and Trp and Glu, variable.

of trace minerals and vitamins were added to the diet as recommended by the NRC¹⁾. The calculated amount of niacin in the diet was not less than 3.5 mg/100 g which was equivalent to 3.5 times the requirement¹⁾. The tryptophan content of the diet was determined electrophotometrically after hydrolysing with barium hydroxide at 130°C for 16 hrs⁹⁾. The stock diet contained 19.0% crude protein.

Plasma amino acid levels. After the feeding experiment, about 0.5 ml of blood was taken from the wing vein of hens using heparinized syringes within 1 hr after oviposition. When the hens had not lay an egg on the day following blood sampling, blood samples were taken again on the following day. Only blood samples from hens which continued to lay for at least 2 consecutive days were used for the determination of amino acids with an automatic amino acid analyzer (Nihon Denshi, JIC-6AH). Plasma, separated by centrifugation at 10⁴g for 10 min as soon as possible, was deproteinized by centrifugation after adding the same volumes of 3% sulfosalicylic acid and stored at -20°C until further analysis.

Experiment 1. The hens were supplied diets with 0.093, 0.125, 0.168, 0.244 and 0.320% of tryptophan, respectively. As the productivity of hens on 0.093% of tryptophan diet decreased sharply within 2 weeks, the hens were switched to the 0.244% tryptophan diet after 2 weeks. After 4 weeks, blood samples were taken within 1 hr after oviposition. In order to confirm the adequacy of diets, the 0.168% tryptophan diet was fed for the following 20 weeks, since on this diet the highest productivity was obtained.

Experiments 2, 3 and 4. To confirm the results obtained in experiment 1, 3 further experiments were conducted. The hens were supplied diets with 0.112, 0.126, 0.141, 0.156 and 0.171% of tryptophan in experiment 2 and 0.086, 0.100, 0.114, 0.128 and 0.156% of tryptophan in experiment 3 and 0.112, 0.141, 0.171, 0.200 and 0.230% of tryptophan in

experiment 4 for 4 weeks, respectively. After the feeding period, blood samples were taken in the same way as in experiment 1.

Statistical method. The significance of differences among treatment means was determined by DUNCAN's multiple range test¹⁰⁾. Multiple regression analysis was used to generate best-fit curves for the changes of tryptophan intake vs all parameters.

Results

Egg production rate (%). As shown in Table 2, the egg production rate of hens on the 0.093% tryptophan diet fell sharply to lower than 50% within 2 weeks, but it recovered to the beginning level within 2 weeks after switching to the 0.244% tryptophan diet. The maximum egg production rate was obtained on 0.168% tryptophan diet. The hens on the 0.168% tryptophan diet maintained an $82.9 \pm 6.3\%$ of egg production for 20 weeks, which was the same rate as that obtained 1 week before the experiment started. Although the

Table 2. Effect of dietary tryptophan levels on egg production rate of laying hens in experiment 1 (%)

Trp(%)	1 w earlier	1 W	2 W	3 W	4 W	Pooled SE
1 0.093	85.7	77.1 ^{a*}	40.0 ^{a***}			
0.244				65.7 ^{a*}	82.9 ^{b*}	3.5
2 0.125	82.9	85.7 ^b	71.4 ^{b***}	77.1 ^{a*}	71.4 ^{a*}	1.3
3 0.168	82.9	82.9 ^b	82.9 ^c	91.4 ^{c*}	85.7 ^c	1.2
4 0.244	82.9	82.9 ^b	88.6 ^{d*}	80.0 ^b	82.8 ^c	1.0
5 0.320	85.7	85.7 ^b	68.6 ^{b***}	68.6 ^{a***}	77.1 ^{b*}	1.0
Pooled SE	2.0	1.2	2.2	2.7	2.2	

Means for 5 hens each. Means with different superscript letters in a vertical row differ significantly at 5% level. Means with asterisks (* and**) in a horizontal row differ significantly at 5% and 1%, respectively, from those of 1 week earlier.

Fig. 1. Egg production rate of laying hens on diets with graded levels of tryptophan.

Means for 5 hens for 2 to 4 weeks.

Expt. 1 -●-, Expt. 2 -○-,

Expt. 3 -■-, Expt. 4 -□-.

egg production rate on diets with 0.125 and 0.320% tryptophan decreased significantly after 2 weeks, during the first week it remained at the same level as during the week before the experiment began. Thus, the data of first week were omitted from statistical treatment in all experiments.

The egg production rate for 2 to 4 weeks in experiments 1 to 4 was plotted against daily tryptophan intake in Fig. 1. The egg production rate increased with increments of dietary tryptophan and then decreased according to the following equation;

$$Y_1 = 3.2 + 0.816X - 0.00194X^2 \quad (r=0.973),$$

where Y_1 and X represent percentage of egg production and mg of tryptophan intake per hen daily. The maximum egg production rate (89.0%) was obtained at 210 mg tryptophan intake/hen/day.

Egg production (g/hen/day). The relationship between tryptophan intake and egg production in experiments 1 to 4 is shown graphically in Fig. 2. The graph of egg production was basically similar to that of the egg production rate, since the egg weight per egg

Fig. 2. Egg production of laying hens on diets with graded levels of tryptophan.

See footnote in Fig. 1.

Fig. 3. Food intake of laying hens on diets with graded levels of tryptophan.

See footnote in Fig. 1.

Fig. 4. Plasma tryptophan concentrations of laying hens on diets with graded levels of tryptophan.

See footnote in Fig. 1.

was not significantly different. The polynomial equation for egg production was $Y_2 = 5.3 + 0.467X - 0.00110X^2$ ($r = 0.963$). This regression equation showed that the maximum egg production (54.9 g/hen/day) was obtained with an intake of 212 mg tryptophan per hen daily.

Food intake (g/hen/day). The response of feed intake was differently influenced by different tryptophan intake (Fig. 3). The equation computed was $Y_3 = 3.9 + 0.687X - 0.00162X^2$ ($r = 0.951$). The point at which daily food intake was maximized (111.6 g/hen/day) on the basis of the regression equation corresponded to be a daily intake of 212 mg tryptophan per hen.

Plasma amino acid concentrations (nmol/ml). As shown in Fig. 4, tryptophan concentrations plotted against daily tryptophan intake per hen increased with increments of tryptophan and then remained relatively constant. The most fitted polynomial equation to the data was $1/Y_4 = 0.0165 - 0.1830/X + 4.19/X^2$ ($r = 0.898$). From this equation the point where the curve changed drastically was not indicative. Thus, the response of plasma amino acid concentration was divided into 2 parts, where plasma amino acid concentration increased sharply according to the equation $Y_5 = 0.360X - 10.5$ ($r = 0.886$) and remained relatively constant according to the equation $Y_6 = -0.003X + 58.8$ ($r = 0.100$). The intersection of both equations was 191 mg tryptophan/hen/day, which was about 11% less than the point which maximized the egg production rate, egg production and food intake.

Body weights. The initial body weights were 1611 ± 13 , 1602 ± 61 , 1661 ± 26 and 1668 ± 16 g in experiments 1 to 4, respectively. Since no significant changes were observed, the data are not reported here. No abnormality was found in any birds throughout the experimental periods.

Discussion

The maximum egg production rate (89.0%), egg production (54.9 g) and feed intake (111.6 g) were observed when the hens consumed 210, 212 and 212 mg tryptophan/hen/day,

respectively. Since 211 mg tryptophan was contained in a 111.6 g diet (ME 3503 kcal), the dietary tryptophan level was computed to be 0.189%. This estimate was higher than those reported by the NRC¹⁾, by HURWITZ and BORNSTEIN (14 mg)¹¹⁾, by TASAKI (0.17%)²⁾ and by MORRIS and WETHLI (0.17%)³⁾. As the 54.9 g of egg contained 112 mg tryptophan ($54.9 \times 12\% \text{ protein} \times 1.7\% \text{ tryptophan}$), the availability of dietary tryptophan for the egg protein was calculated to be 53% (112/211).

The experimental diets contained 3.8 times the niacin recommended by the NRC¹⁾. It is well known that nicotinic acid is synthesized in the animal body from tryptophan, and the nicotinic acid requirement depends upon the tryptophan content of diet. However, the exact extent to which tryptophan helps to meet the nicotinic acid requirement of laying hens under practical conditions has not been established. Conversely, it is not clear the extent to which dietary nicotinic acid levels may spare the tryptophan requirement.

Though there was a small difference in the tryptophan requirement among different strains³⁾, egg production rate and food intake were affected by environmental temperature even in the thermoneutral zone¹²⁾. In this report, about 0.1% tryptophan in the diet was derived from natural feedstuffs, mainly corn and alfalfa meal. It is not clear if the availability of tryptophan in natural feedstuffs and in crystalline are the same. In practice, these factors should be considered carefully in formulating a diet.

Numerous studies^{4,5,8,13-16)} have shown that requirements of many amino acid determined from the plasma amino acid response curve were in close agreement with values obtained using growth rate and nitrogen balance as the criterion in many species. In weanling and adult rats¹⁷⁾, plasma tryptophan levels rose sharply above requirement levels. However, the response of plasma tryptophan concentration to changing dietary tryptophan intake was very unique in young adult men¹⁸⁾ and in pigs¹⁹⁾ as observed here. In the growing pig¹⁹⁾, there was excellent agreement between the dietary tryptophan level at which plasma tryptophan levels reached a plateau and that at which daily weight gain reached a maximum. In the present experiments, as observed in the growing pig, the plasma tryptophan level reached a plateau at 191 mg tryptophan intake/hen/day. This value was lower than that obtained by egg production rate, egg production and feed intake. This difference partly due to the calculation method. In the case of egg production rate, the maximizing tryptophan level was computed as a single point. But in the case of plasma tryptophan concentration, it did not reach a plateau level at the intersection of two equations. As shown by a broken line in Fig. 4, there might be a range (A), which was difficult to be estimated. Only when the range A was narrow, the plasma tryptophan concentration might be useful as a criterion for determination of requirement.

In the case of weanling and adult rats⁶⁾, tryptophan oxigenase activity increased only during the absorptive period as soon as requirements for tryptophan were satisfied, but the increase at suboptimal intakes of tryptophan was not dose related. It is also reported that the degradation rate of tryptophan depends largely on substrate concentration rather than enzyme levels²⁰⁾. But there is too little information to explain a good mechanism to maintain a constant plasma tryptophan level when the dietary tryptophan exceeded the requirement level in contrast to other amino acids.

Summary

1. In order to determine the tryptophan requirement of laying hens, 4 experiments were conducted. Single Comb White Leghorn hens older than 15 months were supplied diets with graded levels of tryptophan from 0.086 to 0.32% *ad libitum*.

2. The egg production rate (Y_1 , %) changed according to the equation $Y_1 = 3.2 + 0.816X - 0.00194X^2$, where X represents mg of daily tryptophan intake/hen. The maximal 89.0% of egg production rate was obtained at 210 mg tryptophan intake/hen/day.

3. The egg production (Y_2 , g/hen/day) changed according to the equation $Y_2 = 5.3 + 0.476X - 0.00110X^2$. The maximal 54.9 g/hen/day of egg production was obtained with an intake of 212 mg tryptophan per hen daily.

4. The equation of the response of food intake (Y_3 , g/hen/day) was $Y_3 = 3.9 + 0.687X - 0.00162X^2$. The point at which the daily food intake was maximized (111.6 g) was computed to be 212 mg tryptophan intake/hen/day.

5. The dietary tryptophan level which maximized the egg production rate, egg production and food intake was calculated to be 0.189%.

6. Plasma tryptophan concentrations increased with dietary tryptophan intakes and then reached a plateau at 191 mg tryptophan intake/hen/day.

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産卵鶏のトリプトファン要求量

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1. 産卵鶏のトリプトファン要求量を求めるため4試験を行った。15カ月齢以上の白色レグホン種にトウモロコシ主体の飼料でトリプトファンが0.086~0.320%のものを自由摂取させた。

2. 産卵率 (Y_1 , %) はトリプトファンの摂取量 (X , mg/羽/日) の増加に伴って次のように変化した。 $Y_1 = 3.2 + 0.816X - 0.00194X^2$ 。トリプトファンの摂取量が210 mg のとき、産卵率は89.0%と最高になった。

3. 産卵 (Y_2 , g/羽/日) は式 $Y_2 = 5.3 + 0.476X - 0.00110X^2$ に従って変化した、トリプトファンの摂取量が

212 mg/羽/日のとき、最高54.9 g/羽/日となった。

4. 飼料摂取量 (Y_3 , g/羽/日) は式 $Y_3 = 3.9 + 0.687X - 0.00162X^2$ に従って変化した、トリプトファンの摂取量が212 mg/羽/日のとき、最高111.6 g/羽/日となった。

5. 産卵率、産卵、飼料摂取量が最高になるときの飼料中のトリプトファンは0.189%であった。

6. 血漿中のトリプトファン濃度はトリプトファン摂取量の増加とともに上昇し、191 mg/羽/日以上では、ほぼ一定の値を示した。

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