Lysine Requirement of Laying Hens

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(Received May 1, 1985)

Abstract 1. To determine the lysine requirement of laying hens, 3 experiments were conducted. White Leghorn hens older than 12 months were fed diets with graded levels of lysine containing 15% crude protein and the same essential amino acid pattern except lysine as in whole egg protein for 4 weeks. 2. Egg production rate (Y_1 , %), egg production (Y_2 , g/hen/day) and feed conversion ratio (Y_3) changed curvilinearly according to the following equations: $Y_1 = -74.4 + 0.451X - 0.000317X^2$, $Y_2 = -15.2 + 0.190X - 0.000131X^2$ and $Y_3 = 4.75 - 0.00775X + 0.00544X^2$, where X was mg of lysine per hen daily. The points where Y_1 , Y_2 and Y_3 reached a maximum or minimum were 711, 725 and 712 mg dietary lysine intake/hen/day. 3. Plasma lysine concentrations remained at relatively low levels until dietary lysine intake increased to 667 mg/hen/day, but thereafter increased rapidly. No effect of dietary lysine was found on the other amino acid concentrations. *Jpn. J. Zootech. Sci.*, 56 (10): 827-834, 1985

Key word: Lysine requirement, Laying hen

Since JOHNSON and FISHER¹⁾ reported the minimal amino acid requirements of laying hens, many studies on the quantitative requirement of dietary lysine of White Leghorn laying hens for the most effective egg production have been reported²⁻¹³⁾. Lysine is essential for laying hens and commonly the first limiting amino acid in commercial diets for laying hens to which a supplement of methionine has been added. Although the NRC¹⁴⁾ recommends a lysine requirement for laying hens of 0.64% of diet, the values reported from 1962 till today have a wide range from 454²⁾ to 920 mg/hen/day¹²⁾.

The lysine requirement of laying hens is not an absolute figure, but is influenced by several factors such as strain¹²⁾, body size¹²⁾, kind of feedstuff³⁾, maximal egg production rate and criteria used for calculating a requirement¹³⁾. The lysine requirement values mentioned above were mainly estimated from egg production rates, in which the hens were supplied the diets with graded levels of lysine. It has been demonstrated that there is a definite relationship between plasma amino acid levels and the amino acid adequacy of the diet. Plasma lysine concentration remains relatively constant at low levels when the dietary lysine levels are less than the requirement and increases in a linear manner when the dietary lysine levels are moderately in excess of that required to maximize growth of chicks¹⁵⁾ and rats¹⁶⁾ and to maximize egg production in laying hens^{13,17)}.

In this paper, we attempted to determine the lysine requirement of laying hens using egg production as the criterion and by the response of plasma lysine concentration to the dietary lysine level.

Materials and Methods

Animals. Single Comb White Leghorn hens (Norin 102) older than 12 months and in higher than 80% egg production were selected and allotted to 5 groups of 5 birds in each experiment. The hens were housed in a single cage in a room with controlled 14 hrs light (5:00-19:00) and were fed the diet and water ad libitum. The weight of eggs produced and feed consumption were recorded at 15:00 every day for 32 days in all experiments. Body weight was checked before and after the experiment.

Diets. Corn-based diets were formulated to provide the same amount of essential amino acids except for lysine and tryptophan as in whole egg protein. They furnished crude protein equivalent to 15% through addition of an amino acid mixture (Table 1). The same amount of trace minerals and vitamins as recommended by the NRC⁽⁴⁾ were added to all diets. In experiments 1 and 2, the lysine content was 0.451, 0.520, 0.590, 0.643 and 0.710%. The tryptophan content was 0.171% in experiment 1 and 0.230% in experiment 2, to investigate the effect of dietary tryptophan level on the lysine requirement. To confirm the results obtained in experiments 1 and 2, in experiment 3, the hens were given diets with 0.50, 0.55, 0.60, 0.65 and 0.70% lysine and 0.171% tryptophan, respectively. All diets were isocaloric and isonitrogenous. The corn-soybean-based stock diet contained 19.0% crude protein and 0.71% lysine.

Plasma amino acid concentrations. After 32 days on experiment about 0.5 ml of blood was taken from the wing vein using heparinized syringes within 1 hr after ovi-

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

	I	II	III
Corn with 2 % fish meal	750	750	750
Alfalfa meal	50	50	50
Calcium carbonate (oyster shell)	60	60	60
Dicalcium phosphate	20	20	20
Mineral and vitamin mixture ¹⁾	6	6	6
Corn oil	30	30	30
Sodium bicarbonate	1	1	1
Agar	2	2	2
Amino acid mixture ²⁾	74.29	74.36	75.05
Tryptophan	1.71	2.30	1.71
Lysine	1.75—4.34	1.75—4.34	2.24—4.24
Glutamic acid	2.59—0	2.59—0	2.00—0
Crude protein (analysis %)	15.1	15.2	15.2
ME (calculation kcal/kg)	2 999	2 999	2 999

¹⁾ 6 g of mixture contained the same amounts of minerals and vitamins as recommended by the NRC⁽⁴⁾. ²⁾ Each amino acid was added until the total amino acid levels in the diet were the same as in 150 whole egg protein, as follows: Arg, 8.56; Met, 4.56; Cys, 3.90; Gly, 5.25; Ile, 8.10; Leu, 12.90; Phe, 7.80; Tyr, 7.05; Val, 9.90; His, 2.68; Pro, 6.75 g/kg and Glu, variable.

position. When the hens did not lay an egg on the next day following blood sampling, blood samplings were repeated again on the following day. Only the blood samples of hens which continued egg production for at least 2 consecutive days were used for the determination of amino acids with an automatic amino acid analyzer (Nihon Denshi, JIC-6 AH). Plasma was separated by centrifugation after adding the same volumes of 3% sulfosalicylic acid and stored at -20°C until further analysis.

Statistical method. The significance of differences among treatment means was determined by DUNCAN's multiple range test according to the methods described by YOSHIDA¹⁸.

Results

Egg production rates. Since the effect of prefeeding diets on the egg production rate lasted for about 4 days, data were gathered for 4 weeks from the 5th to the 32nd day after switching from the stock diet to the experimental diets. As shown in Table 2, the egg production rate increased with increasing dietary lysine intake, reached a maximum level on a 711 mg lysine intake and then decreased. The polynomial equation computed by the method of linear squares was $Y_1 = -74.4 + 0.451 X -$

Table 2. Effects of dietary lysine intake on productivity of laying hens

Trp (%)	Lys (%)	Food intake (g/hen/day)	Lysine intake (mg/hen/day)	Egg production (%) 1 W before 4 W		Egg production (g/hen/day)	Feed conversion ratio
Experiment 1							
0.171	0.451	106.2	479 ^a	94.3	71.4 ^a	49.9 ^a	2.26 ^a
	0.520	105.8	550 ^b	94.3	82.1 ^b	50.6 ^{ab}	2.09 ^{ab}
	0.590	108.4	640 ^c	91.4	88.6 ^b	55.6 ^b	1.95 ^b
	0.643	106.3	684 ^d	91.4	87.1 ^b	55.1 ^b	1.93 ^b
	0.710	107.2	761 ^e	91.4	86.4 ^b	53.6 ^b	2.00 ^{ab}
Pooled SE		1.2	4	3.6	3.2	2.0	0.08
Experiment 2							
0.230	0.451	100.7 ^a	454 ^a	91.4	66.4 ^a	43.7 ^a	2.30 ^a
	0.520	108.1 ^b	562 ^b	94.3	78.5 ^b	49.3 ^b	2.19 ^{ab}
	0.590	105.9 ^b	625 ^c	91.4	83.6 ^b	51.5 ^b	2.06 ^b
	0.643	107.7 ^b	693 ^d	94.3	87.1 ^b	53.3 ^b	2.02 ^b
	0.710	108.4 ^b	770 ^e	94.3	84.8 ^b	52.5 ^b	2.06 ^b
Pooled SE		1.1	5	3.6	2.9	1.4	0.07
Experiment 3							
0.171	0.495	104.8 ^{ab}	519 ^a	94.3	68.6 ^a	45.3 ^a	2.31 ^a
	0.547	103.0 ^a	564 ^b	91.4	76.4 ^{ab}	48.0 ^{ab}	2.15 ^{ab}
	0.594	108.9 ^c	644 ^c	91.4	83.6 ^b	54.0 ^b	2.02 ^{ab}
	0.643	107.1 ^{bc}	689 ^d	94.3	85.0 ^b	55.1 ^b	1.94 ^b
	0.691	109.6 ^c	757 ^e	94.3	83.6 ^b	54.3 ^b	2.02 ^{ab}
Pooled SE		1.0	4	3.6	3.0	2.3	0.10

Means for 5 hens each. Values in the same column with different superscript letters differ significantly at the 5% level.

Fig. 1. Effects of dietary lysine intake on egg production rate in experiment 1 (●), 2 (○) and 3 (□).

$0.000317 X^2$ ($r=0.899$), where Y_1 and X represent percentage of egg production and mg of lysine per hen daily. The curvilinear response curve based on this equation and a plot of the data observed against the dietary lysine intake are shown in Fig. 1. The point at which egg production rate maximized (86.4%) on the basis of the regression equation corresponded to a daily intake of 711 mg lysine per hen.

Egg production. Since the weight of each egg changed within a narrow range during the experimental periods, a similar polynomial equation was fit to the egg production data. The polynomial equation for egg weight was $Y_2 = -15.2 + 0.190 X - 0.000131 X^2$ ($r=0.930$). This regression equation showed that maximum egg production (53.8 g) was obtained with an intake of 725 mg lysine per hen daily. This estimation was 2% higher than that by egg production rate.

Feed intake. When the dietary lysine levels were lower than 0.547%, feed intake decreased, but no dose-related response was found at higher dietary lysine levels.

Feed conversion ratio. The response of feed conversion ratio was influenced by different dietary lysine intakes with a curvilinear equation, $Y_3 = 4.75 - 0.00775 X + 0.00544 X^2$ ($r=0.950$). The minimal feed conversion ratio was attained at 712 mg lysine intake, which agreed very well with that of egg production rate.

Body weight. The initial body weight of laying hens was 1656 ± 22 , 1649 ± 33 and 1677 ± 32 in experiments 1, 2 and 3, respectively. Since no significant changes were observed during the experimental periods, the data are not presented here. No abnormality was found in any birds except 1 and 2 hens on the 0.415% lysine diet in experiments 1 and 2, respectively, molted lightly during the 4th week.

Plasma amino acid concentration. The results for plasma concentrations of tryptophan, lysine, arginine and phenylalanine are shown in Table 3. The concentrations of other amino acids measured are not presented here, since they were not affected by the dietary lysine intakes as were arginine and phenylalanine. The plasma tryptophan concentration remained constant at all dietary lysine intake levels at both dietary tryptophan levels. But lysine concentrations on the 0.171% tryptophan diet

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Table 3. Effects of dietary lysine levels on plasma amino acid concentrations

Trp (% of diet)	Lys	Plasma amino acids (nmol/ml)			
		Trp	Lys	Arg	Phe
Experiment 1					
0.171	0.451	50	195 ^a	244	91
	0.520	54	207 ^a	272	88
	0.590	50	218 ^a	244	74
	0.643	60	274 ^b	290	95
	0.710	52	421 ^c	252	94
	Pooled SE	5	10	11	5
Experiment 2					
0.230	0.451	64	161 ^a	258	99
	0.521	59	178 ^a	239	86
	0.590	57	188 ^a	269	80
	0.643	56	245 ^b	241	98
	0.710	59	405 ^c	252	103
	Pooled SE	3	9	13	4
Experiment 3					
0.171	0.495	50	180 ^a	260	98
	0.547	58	196 ^a	237	97
	0.594	47	204 ^a	282	105
	0.643	56	248 ^b	292	102
	0.691	58	437 ^c	254	103
	Pooled SE	3	10	11	3

See footnotes in Table 1.

Fig. 2. Effects of dietary lysine intake on plasma lysine concentrations in experiment 1 (●), 2 (○) and 3 (□).

(experiments 1 and 2) were always higher than those on the 0.230% tryptophan diet (experiment 2).

As shown in Fig. 2, the plasma lysine concentration increased very slowly up to

a 667 mg lysine intake per hen daily ($Y_4=0.17X+99$, $r=0.657$). Above that level, the plasma lysine concentration increased rapidly according to the equation, $Y_5=2.16X-1228$ ($r=0.962$), where Y and X represent nmol/ml of lysine and mg of dietary lysine intake, respectively. The requirement point from both equations was estimated to be 667 mg/hen daily. This value was 6% less than that estimated from the maximum egg production rate.

Discussion

The requirement of lysine for maximal productivity obtained from egg production rate, egg production, feed conversion ratio and plasma lysine response were 711, 725, 712 and 667 mg/hen daily. These values were higher than those of MORAN *et al.*²⁾ (454), HARM and WALDROUP³⁾ (494), BRAY⁴⁾ (520), LASTSHAW⁵⁾ (585-650), SELL and HODGSON⁷⁾ (620-660) and SHELL and JOHNSON⁸⁾ (625), but agree with those of HURWITZ and BORNSTEIN⁶⁾ (627-836) and JENSEN *et al.*¹¹⁾ (666-788). Higher dietary lysine requirements have been reported for wheat-based rations. MARCH and BIELY¹⁰⁾ stated that hens fed wheat-based rations required 800 to 850 mg of lysine daily. Similarly, JENSEN *et al.*¹¹⁾ and PILBROW and MORRIS¹²⁾ reported that 765 to 900 mg lysine per hen daily were needed to support maximum egg production. On the other hand, SHELL and HODGSON⁷⁾ and SHELL and JOHNSON⁸⁾ found that 620 to 660 mg lysine from wheat-based diets satisfied the daily requirement of hens. These differences might be partly due to differences in availability of lysine in feedstuffs, because the availability of corn is higher (85-90%) than that of wheat (75%) (YAMAZAKI, unpublished data).

In the 0.643% lysine diet, 0.176, 0.063, 0.037 and 0.367% of lysine were derived respectively from corn, fish meal, alfalfa and crystalline lysine. Assuming an availability of lysine in corn is 86%¹⁹⁾ and 85% in fish meal and alfalfa and 100% in crystalline lysine, the availability of lysine of this diet might not be less than 94%. Since the maximum 53.8 g of egg was obtained at a 725 mg dietary lysine intake, and since 426 mg lysine was present in 53.8 g egg, the availability of absorbed lysine for egg formation would be estimated to be 63.9% ($426/725 \times 0.92$). Since hens used for maintenance 66 mg lysine/hen daily ($40 \text{ mg/kg/day for maintenance}^{20)} \times 1.66 \text{ kg average body weight in experiments 1 to 3}$), the true availability of absorbed lysine might be estimated to be 75.0% ($(426+66)/713 \times 0.92$).

As the hens on 0.590 and 0.643% lysine diets consumed 107.4 g/day, the diet for maximal egg production rate should contain approximately 0.66% of lysine (711 mg/107.4 g), if the availability of lysine in feedstuffs was the same as in this experiment diet, which was 3% higher than the NRC¹⁴⁾ recommendation.

The response of plasma lysine concentration could be used to estimate the dietary requirement of lysine for laying hens as in chicks¹⁶⁾ and rats¹³⁻¹⁷⁾. As CHI and SPEERS^{13,17)} reported, when hens were fed a diet containing varying amounts of lysine, plasma lysine remained at relatively low levels until dietary lysine intake increased to 667 mg/hen/day, but thereafter increased rapidly. In contrast to the observations by CHI and SPEERS¹⁷⁾, no significant response to the dietary lysine intake was found for other

amino acids. The decrease in the plasma arginine level with an increase of dietary lysine has been shown in chicks²¹⁻²²⁾ and laying hens¹⁹⁾. However, as shown in Table 3, the plasma arginine concentration was not changed with a trend corresponding to the dietary intake levels. The plasma arginine concentration was reduced only in hens fed a diet containing 0.85% lysine, suggesting that the lysine levels employed here were too low to induce the lysine-arginine antagonistic relationship in laying hens.

Acknowledgements

The author is grateful to Dr. H. FISHER, of Rutgers University, for his reading of the manuscript and to Kyowa Hakko Kogyo Co., Ltd. and Takeda Feed Supplement Co., Ltd. for generously supplying crystalline amino acids and vitamin-mineral premix.

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産卵鶏のリジン要求量

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1. 産卵鶏のリジン要求量を求めるため, 12 カ月齢以上の産卵鶏を用いて, 3 試験を行なった. トウモロコシ主体の飼料にリジンとトリプトファン以外は 15% 全卵蛋白質と同じになるように結晶アミノ酸混合物を添加した.

2. 試験飼料を 4 週間自由摂取させた結果から, リジン摂取量 (X , mg/羽/日) に対し,

$$\text{産卵率 } Y_1 (\%) = -74.4 + 0.451 X - 0.000317 X^2$$

$$\text{産卵重量 } Y_2 (\text{g/羽/日}) = -15.2 + 0.190 X - 0.000$$

$$131 X^2$$

飼料要求率 $Y_a = 4.75 - 0.00775 X + 0.00544 X^2$ という回帰式が得られた. それぞれの式から, 要求量は 711, 725, 712 mg/羽/日と計算された.

3. 血漿中のリジン濃度は 667 mg/羽/日 まではゆっくり増加し, その後は急に増加した. ここで用いた飼料中のリジン量では他のアミノ酸の血漿中の濃度に影響はみられなかった.

日畜会報, 56 (10): 827-834, 1985