VARIATION AND SELECTION FOR HIGH AND LOW LYSINE DEPENDENT RATS

by

SAMAD SHIEHZADEH

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Approved by:

Lowell H Harbers
Major Professor

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INTRODUCTION

Lysine is one of the essential amino acids that is required for maintenance and growth of farm animals. The level of this amino acid is relatively low in most of the commonly used feed-stuffs. The feeds which are rich in lysine are costly and in short supply.

It has been shown that lysine utilization is inheritable in poultry (Griminger and Fisher, 1962). Enos et al. (1966) reported that the efficiency of lysine utilization is a genetically controlled trait. Goodman (1956) reported strain differences amoung rats in utilization of certain amino acids. A preliminary experiment with a lysine deficient diet in our laboratory resulted in a random variation in the growth rate of rats. This finding was the basis for the following experiment in investigating the possibility of producing lines of rats that differ in their efficiency for growth and feed utilization on a lysine deficient diet. Because of its relatively fast generation interval, ease of handling and low cost of maintenance the albino rat was chosen as the experimental animal. Hopefully these findings would be useful for later application to the feeding and breeding of monogastric livestock species and especially swine, because of nutritional similarities between that animal and rats. Apart from this, studies of this nature and investigation of genetic variations in utilization of nutrients will help in solving the nutritional problems with more insight and will facilitate interpretation of discrepancies in feeding trials.

LITERATURE REVIEW

Lysine has been found to be indispensible for growth and maintenance of rats, (Osborne and Mendel, 1914) chickens, pigs and dogs (Almquist, 1956). Neuberger and Webster (1945) showed that the need for maintenance is much less intense than that for growth. Requirement of lysine for maintenance in adult rats was reported by Benditt et al. (1950). In this study it was shown that lysine requirement was 50 mg. per kg. per day for nitrogen balance and 59 mg. per kg. per day for maintenance of body weight. Mitchell (1950) reported the same values. Almquist (1956) in his studies concluded that 30% of the optimal amount of lysine is the maintenance value.

The lysine requirement for growth of rats has been reported by Rose et al. (1949) and Bressani and Mertz (1958). Rose estimated the requirement for growth of the rat at 1.0% of the diet. In terms of grams lysine per 16 gm. of nitrogen, the requirement would be 8.1 gm. It has been reported by Almquist (1956) that the lysine requirements for chicken and pig depends on the protein level of the diet. At higher levels of protein, growth was better thus more lysine was required because more tissue was formed.

Lysine deficiency has been found to result from a general inhibition of protein synthesis. The major symtoms of deficiency includes cessation of growth, muscle wasting, hypoproteinemia and anemia. Harris and associates (1943) and Gillespie et al. (1945) studied lysine deficiency in rats. Along with other symptoms reduction of calcification of bones was noted.

Lysine deficiency has been shown to cause corneal vascularization in rats (Sydenstricker et al., 1946) and a microcytic hypochromic anemia in chickens (Klain et al., 1957). Fat deposition in rat tibia has been reported to be associated with lysine deficiency (Rohdenburg, 1958). This finding has been confirmed by Braham et al. (1960) in chickens. Likins et al. (1957) reported that lysine deficiency resulted in a decrease in the skeletal deposition of radiocalcium, apparently caused by a failure in bone development. Vohra and Kratzer (1956) reported that lysine deficiency in growing black rats specifically causes graying of hair. Fatty livers also result from lysine deficiency (Signal et al., 1953). Sidransky and Baba (1960) have studied morphologic and biochemical changes in young rats force-fed diets devoid of valine or lysine. Deficiency of either caused fatty liver, excess liver glycogen and atrophy of the pancreas, parotid gland, thymus and spleen within six days. Protein content of liver was unaffected. Young rats fed the deficient diet ad libitum, in contrast with those force-fed, showed a loss of muscle and liver protein as well as a decreased liver lipid and glycogen.

Using rats, Gullino et al., (1956), have studied the toxicity of the individual and mixtures of essential amino acids. The least toxic amino acid was isoleucine and the most toxic was tryptophan. D-lysine and L-lysine had essentially the same toxicity effect. The LD_{50} dose for L-lysine was 3.2 gm./kg. of body weight. The effect of ingestion of a large excess of lysine along with food also has been studied. Russell et al., (1952) fed rats a basal diet containing 10% casein. When 5% of DL-lysine was added to this diet the weight gain in 10 days was 56% of that of the control group. This diet furnished fourfold excess lysine over the control. Phillips and Berg (1954) have shown that the use of

twice the level of DL-lysine in the diet does not interfere with growth. Recently Harris and Burress (1959) studied the effect of feeding five to eightfold excess of lysine. Four week weight gains were only slightly reduced below those observed with optimal supplementation and toxic symptoms were observed.

Lysine has a unique position in the general scheme of amino acid metabolism. Lysine apparently does not participate in reversible transamination reaction in the body. Lysine, labeled with deuterium and N^{15}, is incorporated into protein in vivo without appreciable change in the $D:N^{15}$ ratio. When N^{15} amino acids are given to rats no incorporation of N^{15} into alpha-amino groups of lysine occurs. Also, only slight labeling of the alpha-hydrogen atom of lysine occurs in the intact rat (Meister, 1957). Lysine is irreversibly transformed in the body, via pipicolic acid and alpha-amino adipic acid to glutaric acid (Greenberg and Rothstein, 1959). The glutaric acid may be oxidized to alpha-keto-glutaric acid and then enter the tricarboxylic acid cycle. The nitrogen loss by such irreversible deamination presumably can enter the body pool of nitrogen and be used in part for the synthesis of nonessential amino acids.

Genetic variation in utilization of essential amino acids

Evidence that efficiency of feed utilization is inherited was reported by Hess et al. (1961). McCartney and Jull (1948) observed differences in feed utilization to 10 weeks of age between two different strains in New Hampshire chickens. Thornton and Whittet (1960) presented evidence that commercial strains of layers had slightly different protein requirements. Moreng et al. (1964) reported variation in ability of growth amoung four commercial strains as measured by their response to three different levels

of dietary protein. Biely and March (1961) concluded that birds of different genetic background may differ in their amino acid requirements for production. Hess et al. (1960) successfully demonstrated growth rate differences for fast and slow growing lines of chickens on methionine deficient diets. Griminger and Fisher (1962), experimenting with chickens, showed significant differences among dams in growth potential of their offspring on arginine and lysine deficient diets. Enos (1965) demonstrated that progeny of different sires exhibited differences for lysine utilization. Lepore (1965) reported that his selection for fast and slow growth of chickens to three weeks of age on a diet deficient in methionine did not differentiate two lines differing in requirements for that amino acid. Hegested et al. (1941) has reported differences between breeds of chicks in their requirements for arginine. Griminger and Fisher (1962) observed differences in growth rate amoung chicks from certain hens when fed diets deficient in either lysine or arginine. These investigators indicated that the variation due to deficiency of lysine was somewhat less than that observed with deficiency of arginine. This has been supported by the findings of Neshiem and Hutt (1962) who reported that variation in response to a deficiency of lysine within and amoung the three strains originally studied was much lower than for arginine. In a later report, Hutt and Nesheim (1966) concluded that the differences between the two lines presumably is the result of changed efficiency of metabolism of arginine. It was further stated that "It is very unlikely that the low requirement line has developed the ability to synthesize arginine." These investigators carried out their selection for four generations. Tests in the fourth generation showed that amoung 277 chicks of high requirement line, mortality was 27.8%, average weight of survivors 110.5

gm., and survivors attained only 36.4% of the average weight of control. In the low requirement line, mortality was only 1.8% and the average weight of survivors was 258.4 gm. (83.3% weight of control). Data presented by Jones et al. (1966) suggested that lysine-arginine metabolism in rats is quite similar to that described in chicks. However, the chick's ability to synthesize arginine introduces differences in the data obtained in the two species. Goodman (1956) reported strain differences amoung mice in utilization of optical isomers of certain amino acids. However, no study on the selection for lysine in rats has been reported.

EXPERIMENTAL PROCEDURE

Animals and Management: Sprague Dawley rats were individually housed in screen bottomed cages in an air-conditioned room maintained at 75°F with 16 hours of light each day. The rats were handled uniformly during each section of the experiment. All diets were fed ad libitum with water continuously available.

Experimental Diet: The composition of the experimental diet is shown in Table 1. This diet was suggested by Hawk et al.,(1954) as lysine deficient and would cause poor growth when fed to young rats. The amino acid composition of the diet, found by amino acid analysis using a Beckman analyzer, is shown in Table 2.

Experimental Designs: In generation zero a group of 33 weanling rats ranging in weight from 45-59 gm. were weighed, marked, sexed, caged, and fed the experimental diet. Rats were weighed daily for 31 days. At the end of this period, differences in weight gain became apparent. To study the amino acid composition of the urine for metabolic differences between the rapid and slow gainers, eight rats were transferred to metabolic cages where 24-hour urine samples were collected. During, and two hours before the urine collection, rats were deprived of feed; only water was available.

The animals were then divided into three groups. Rats with the largest gain were considered to have a low lysine requirement and were labeled LL. Low gaining rats, i.e., those needing high lysine were labeled HL, and those intermediate, ML. Two males and two females from each group

were mated. Animals were maintained on a commercial diet (Purina Laboratory chow) during breeding, gestation and lactation. Pups were weaned at 25 days of age, and used for study as the first generation.

In the first generation, a group of 52 rats were used for growth differentiation, as previously described. In addition feed consumption was determined at 10 day intervals. During the last four days, known amounts of feed were given daily and feces were collected for proximate analysis. Data obtained were used for feed efficiency and feed utilization. Possitive assortive matings were used in the first generation on the basis of body weight gain and feed efficiency.

Thirty-three pups were used to study second generation responses. Laboratory management procedures during breeding, gestation and lactation were described earlier. Data on growth and feed efficiency were determined by a 31-day feeding trial. Blood samples from 6 rats (3 LL and 3 HL) were taken for amino acid analysis to study group differences, particularly in lysine metabolism.

TABLE 1. Composition of the Experimental Diet

Ingredient	%
Oat Meal	60
Corn Starch	30
Hegested salt mixture	4
Mazola Oil	5
Cod Liver Oil	1

TABLE 2. Amino Acid Composition of Lysine Deficient Diet

	Amino Acid	Gm. Amino Acid per 100 gm. sample	Mole Amino Acid per 100 moles
1.	Lysine	0.345	2.661
2.	Histidine	0.210	1.529
3.	Arginine	0.700	4.533
4.	Aspartic acid	0.793	6.721
5.	Threonine	0.328	3.104
6.	Serine	0.474	5.094
7.	Glutamic acid	2.351	18.042
8.	Proline	0.525	5.153
9.	Glycine	0.451	6.771
10.	Alanine	0.443	5.606
11.	Half Cystine	0.414	1.944
12.	Valine	0.508	4.896
13.	Methionine	0.141	1.066
14.	Isoleucine	0.361	3.106
15.	Leucine	0.755	6.494
16.	Tyrosine	0.364	2.270
17.	Phenylalanine	0.515	3.156
18.	Ammonia	0.263	17.494

RESULTS AND DISCUSSION

Diet: The experimental diet contained 10% protein, which is half of the amount necessary for optimum growth. Its essential amino acid content is above maintenance level but not sufficient to insure maximum growth of rats. The amount of lysine in the diet was lower than that of any other amino acid; while its requirement for growth is higher. Furthermore, approximately 20% of the lysine is biologically unavailable to the rat. Table 2 shows that the lysine content of the diet was 0.345% or 34.5% of the optimal amount. This diet, therefore, was used to place the rats under lysine stress without causing lysine or protein deficiency symptoms.

Zero Generation: The results of the zero generation showed that in spite of the essentiality of lysine, its requirement for growing rats was highly variable. When weanling rats from a stock population (zero generation) were fed the low lysine diet over a period of 31 days; a wide range of variation in the utilization of lysine was expressed by individual rats, as manifested by a significant difference in the rate of growth. Table 3 shows the range, the mean and the standard deviation for growth of the control and the experimental population.

TABLE 3. Mean and Standard Deviation of Gain for Control and Experimental Population

Population	Number of Rats	Range of Gain	Mean Gm.	Standard Deviation
Experimental	29	14-79	48.68	14.88
Control	4	96-106	102.00	3.74

The weight gain ranged from 14 gm. to 79 gm. and 96 gm. to 106 gm. for experimental and control groups, respectively. The mean for the experimental group was 48.68 gm. with a standard deviation of 14.88. The mean for the control group was 102 gm. and a standard deviation of 3.74. The low protein level of the experimental diet was obviously the main factor responsible for the suppressed growth. The variation in the rate of growth could be attributed to the low content of lysine (the level of other essential amino acids was high for a 10% protein level). It is, therefore, reasonable to assume that the variation of the growth is due to the ability of some individual rats to utilize lysine more efficiently than others. If there are some other nutritional elements which individually or collectively caused this difference it would be interesting by itself. This could be verified by using a purified diet. Enos et al. (1965) observed a similar difference in the rate of growth between control and lysine deficient groups of chickens. The extremes of range for individual gain response as a percent of control was 9.3 to 72.16% indicating certain chickens had greater or lesser abilities for lysine utilization. Griminger and Fisher (1962) observed considerable variation in body weight when chickens were fed a diet containing half

the quantity of lysine which is considered necessary for optimum growth. These results showed an inherited growth potential for chicken given a lysine deficient diet.

Urinary amino acid analysis (Table 4) revealed no significant differences amoung individuals with regards to lysine excretion. This indicates that the difference in lysine utilization does not have any effect on lysine output through urine.

TABLE 4. Urinary Lysine Excretion of High (HL), Low (LL) and Intermediate (ML) lysine-dependent Rats from Generation Zero

Rat ID	2	3	5	6	12	17	18	28
Group Designation	HL	HL	LL	LL	ML	ML	HL	HL
Gram lysine per 100 gm. urine	25	30	28	29	19	27	30	27

First Generation: The rats of the zero generation were divided into three groups. The rats that grew well were selected for LL (Low-Lysine) line, while those that grew poorly were selected for HL (High-Lysine) line. The intermediates were selected as (ML) line (Table 5).

TABLE 5. Initial, 10-days Interval and Net Gain of Rats Selected for Mating from the Zero Generation

Rat ID F=Female M=Male	Line	Initial Weight	Weight at 10 days	Weight at 20 days	Weight at 30 days	Net Gain
2 M	HL	59	72	82	100	41
3 M	HL	50	61	70	90	40
5 M	LL	51	79	95	115	64
6 M	LL	58	76	93	137	79
8 M	ML	54	70	87	107	53
12 M	ML	55	71	77	105	50
17 F	ML	54	73	80	104	50
18 F	HL	51	61	67	86	35
23 F	LL	52	57	82	115	63
26 F	LL	54	76	80	114	60
27 F	HL	54	69	71	88	34
31 F	ML	54	67	88	112	58

Two males and two females from each group, LL, HL and ML were mated (Table 6). Fifty-two rats were thus produced which were tested as selected first generation. They were put on experimental diets for a period of 31 days. They were treated as previously explained. The mean of weight gain, feed consumption and feed efficiency ratio is shown in Table 7.

TABLE 6. Mating of Zero Generation Rats According to Weight Gain

LL X LL Rat ID		ML X ML Rat ID		HL X HL Rat ID	
M	F	M	F	M	F
5	23	8	17	2	18
6	26	12	31	3	27

The result showed that the population in the first generation is well divided into two distinct groups with regard to the weight gain and feed efficiency ($P < .05$). The mean of weight gain for offspring of LL line was 53.76 gm. while that of HL group was 40.10 gm. The mean weight gain and feed efficiency of ML group was smaller than that of the LL and greater than HL group. This significant difference between the two lines indicates that the ability to utilize the low lysine diet is inheritable.

TABLE 7. The Mean of Body Weight Gain, Feed Consumption, and Feed Efficiency Ratio of LL and HL Lines of the First and the Second Generation and ML Line of the First Generation

Generation	Line	Number of Individuals	Mean Gm. Gain	Mean Feed Consumption	Mean Feed Efficiency
1	LL	17	53.76	265.94	4.94
1	HL	19	40.10	381.47	9.48
1	ML	16	49.62	321.51	6.52
2	LL	22	60.63	315.95	5.17
2	HL	11	41.63	326.45	7.84

A digestibility trial was performed on the feed and feces of nine rats from both groups (Table 8). The purpose of this study was to determine whether there is any difference in the digestion of the feed by the individuals of the two groups. This trial showed that digestibility for dry matter and organic matter was similar. There was no significant difference in digestible energy between the two groups. However, there was some difference between the two groups with regards to the protein digestibility and retention. Protein digestibility was 4.8 percent greater for the LL group. When based on metabolic body size the LL group consumed 17.09 percent more protein and retained 21.03 percent more of this protein than the HL group. The high weight gain by the LL group could not totally result from the difference in digestibility.

TABLE 8. The Mean of the Dry Matter, Organic Matter, Digestible Energy, Apparent Protein Digestibility and Retained Protein of LL and HL Rats of the First Generation

	Digestibility Coefficient (%)					
Group of Rats	Dry Matter	Organic Matter	Digestible Energy	Protein	Protein Retained	Gm. Protein Retained, $Kg._W.^{75}$
LL	91.52	94.25	91.52	80.22	3.42	81.52
HL	91.62	93.13	91.62	75.81	2.8	67.35

Second Generation: To produce the second generation, four rats (two males and two females) from each LL and HL group were selected for positive assortive matings (Table 9) on the basis of weight gain and feed efficiency.

TABLE 9. Mating of the First Generation

LL X LL							HL X HL						
Male	Sire No.	Dam No.	X	Female	Sire No.	Dam No.	Male	Sire No.	Dam No.	X	Female	Sire No.	Dam No.
37	5	23	X	36	5	23	2	3	27	X	44	2	18
52	6	26	X	49	6	26	2	3	27	X	46	2	18

The weight gain and feed efficiency of the selected individual for mating from the first generation is shown in Table 10.

TABLE 10. Final Weight Gain and Feed Efficiency of Selected Rats for Mating from the First Generation

Rat ID	Group Designation	Weight Gain	Feed Efficiency
2 M	HL	28	6.40
36 F	LL	53	6.14
37 M	LL	61	4.78
44 F	HL	40	11.0
46 F	HL	22	9.65
49 F	LL	55	4.36
52 M	LL	52	4.54

The two lines in this generation exhibited some differences in weight gain and feed efficiency. This difference was statistically significant ($P < .05$). As it is shown in Table 7, the mean weight gain of the LL group was higher than that for the HL group.

The average gain of the LL group of the first generation was lower than that of the second generation. This shows than an improvement has been made in the second generation by LL line. A change in the feed efficiency in the opposite direction was shown by both groups. The reason for the lack of improvement in feed efficiency was that the emphasis on selection was on the weight gain rather than feed efficiency.

Amino acid analysis on the pooled plasma of three individuals from each LL and HL group showed that lysine content of plasma of the LL group was 191 mg. lysine per 100 gm. protein and that for the HL group was 525 mg. lysine per 100 gm. protein. The histidine content of the HL group was slightly higher than that for the LL group. On the other hand the arginine content of plasma of LL group is higher than HL group. The low content of lysine in the plasma of LL group indicates that the LL individuals have a greater ability in incorporating lysine into the body tissue.

The results of the three generations studied showed that two lines in each generation are distinguishable as far as weight gain and feed efficiency. Feed consumption in either generation was higher for HL line while LL line gained more. There was a significant improvement in the LL line of the second generation. The HL line in the second generation did not change significantly.

Weight gain and feed efficiency are quantitative traits, the inheritance of which is controlled by many genes. Less than 50% of the variation that exists amoung individuals is actually attributable to genetics. This indicates that environment plays a significant role in the expression of the weight gain and feed efficiency traits. In this study there were some changes in handling of rats such as age at which the rats were put on the experimental diet and feed preparation. This inconsistency may have

had some effect on the results of the experiment. The fact is that utilization of the low lysine diet was variable and this variation was expressed in each generation. The separation of the two lines in each generation indicates that this variation in lysine utilization is inheritable.

SUMMARY

This experiment was designed to study the effect of selection for growth and feed efficiency of rats fed a lysine deficient diet. The diet consisted of oat meal, starch, mazola oil, cod liver oil and a salt mixture. Twenty-nine albino rats were tested as the base population for a period of 31 days during which water and feed were given ad libitum. A large variation in the rate of growth became evident at the end of this period which was attributed to the differences in the ability of rats to utilize the deficient diet. Positive assortive matings were made on the basis of the growth rate.

Rats which grew well were assumed to have utilized their feed more efficiently and thus were labeled low lysine line (LL), those which grew poorly were considered high lysine line (HL) and those intermediate, (ML). Two males were mated to two females in each group and their progeny was tested as the first selected population. In the first generation there was a significant difference ($P < .05$) between the LL and HL groups with regards to growth rate and feed efficiency. The average weight gain for LL, HL and ML groups was 53.76 gm., 40.1 gm. and 49.63., respectively. From the LL group four superior rats, two males and two females, were selected for mating and from the HL group, four inferior rats were mated and their offspring were tested as the second selected population. In the second generation the means of the feed efficiency and weight gain of the LL group were significantly different ($P < .05$) from that of the HL group. An amino acid analysis on the urine of the individuals of the two

groups revealed no difference in lysine output through the urine. However, the lysine content of the plasma of HL group was significantly higher than that of the LL group. A digestibility trial showed that dry matter, organic matter and digestible energy were similar for the two groups. However, the protein digestibility and retention were higher for the LL group.

The results of the three generations indicated that rats differ in their ability to utilize the lysine deficient diet and this difference was inheritable. In each generation there was a significant difference between lines with respect to feed efficiency and weight gain. An improvement in the rate of growth and feed efficiency was shown by the LL line ($P < .05$) for each successive generation, but none for the HL line after the first generation.

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This experiment was performed on three generations of rats to study the possibility of selection for the utilization of a low lysine diet. Selection in the zero generation was based on the body weight gain over a period of 31 days on the lysine deficient diet. The rats which grew well were assumed to have a greater ability to utilize the low lysine diet; therefore were selected for low lysine line (LL). The poor growers were selected for high lysine line (HL). Two males and two females from each LL and HL group were mated to produce the first selected generation. In the first generation there was a significant difference in weight gain and feed efficiency between the offspring of the LL and HL group ($P < .05$). The average weight gain for LL group was 53.76 gm. and that for the HL group was 40.10 gm. The feed efficiency for the LL and HL groups were 4.94 and 9.48, respectively. Urine analysis showed no difference in amino acid excretion.

To produce the second selected generation the same procedure of selection and mating was performed as for the first generation. Thirty-three rats were thus produced which were tested for weight gain and feed efficiency. The difference between the LL and HL groups with regard to both feed efficiency and weight gain was significant ($P < .05$). The average weight gain for LL group was 60.63 gm. and that for HL group was 41.63 gm. The feed efficiency for LL and HL groups was 5.17 and 7.84, respectively.

When the average gain of the LL group of the first generation was compared with that of the second generation a significant improvement in selection became evident. However, the HL group did not show any significant difference or improvement. A change in feed efficiency in opposite direction was shown by both groups. The reason for the lack

of improvement in feed efficiency was that the emphasis in selection was on the weight gain rather than feed efficiency.

A digestibility trial was carried out on the LL and HL group of the first generation. Dry matter, organic matter, and energy digestibility were similar for both groups. However, there were some differences between the two groups with regard to protein digestibility and protein retained (based on Kg. $W^{.75}$).

Amino acid analysis on the pool plasma of 3 individuals from each LL and HL groups showed that lysine content of plasma of LL group was 191 mg. lysine per 100 gm. protein and that for HL group was 525 mg. The low content of lysine in the plasma of LL group indicated that the LL individuals have greater ability in incorporating lysine into the body protein.