Methionine and Lysine Supplementation of Animal Feeds

HANS R. ROSENBERG
Stine Laboratory, E. I. du Pont de Nemours & Co., Inc., Newark, Del.

Methionine or lysine is the first limiting essential amino acid in commercial mixed feeds. Successful supplementation of a feed consists of adding the first limiting essential amino acids to the feed in such a manner as to achieve a balance with the second limiting essential amino acids as any amount in excess of that needed for proper balance is lost. Whether or not all of the first limiting essential amino acids needed to obtain balance can be utilized depends upon the energy content of the diet which regulates feed consumption.

THE AMINO ACID most likely to be first limiting in the protein of commercially produced feeds is either methionine or lysine. To help alleviate amino acid imbalance in these feeds the chemical industry has spent much effort to devise economic processes for the production of synthetic methionine and lysine. However, as these became commercially available a lack of fundamental knowledge concerning their requirements and utilization became apparent.

Tables on the requirements of all nutrients, including amino acids, for various species of domestic animals, as well as for man, have been compiled by the National Research Council (*13*). However, many values for amino acid requirements are not available. A complete list is given only for the growing chick, and the National Research Council specifies not only the requirements for all essential amino acids but also for total protein. These National Research Council requirements are summarized diagrammatically in Figure 1. The descending line represents the 20% protein level specified by the National Research Council and the ordinate is provided with a scale to measure the amounts of amino acids as per cent of the diet. Bars representing the requirement for each essential amino acid according to the National Research Council value are fitted on the abscissa under the descending line. For example, the bar representing lysine reaches the requirement line at the 1.0% level, signifying that this amount of lysine in the diet represents the National Research Council requirement figure.

The array of essential amino acids under the 20% protein line in Figure 1 may be called the pattern of the chick's essential amino acid requirements. If all essential amino acids in a diet are present in the ratios indicated by this pattern, its protein can be termed balanced or ideal. Similar requirement patterns can be drawn for other species of animals.

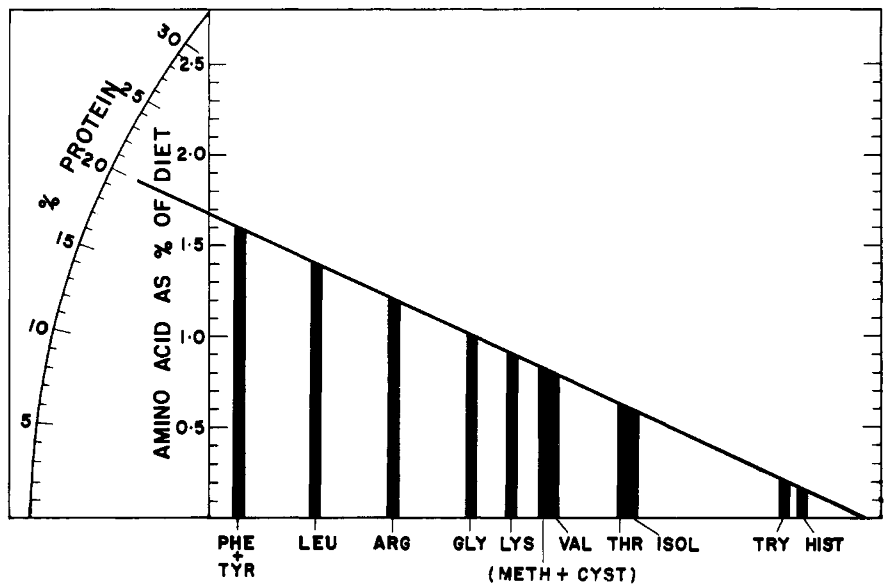

Figure 1. Essential amino acid requirements of the growing chick

To obtain the essential amino acid pattern of mixed feeds, the amounts of the individual amino acids are calculated from the amino acid composition of the ingredients. These are either taken from one of the published feed composition tables or determined by analysis. Although the values obtained in this manner do not always agree with biological assays, they have proved to be useful.

The total of all of the essential amino acids required by the growing chick, according to the National Research Council, amounts to 9.25%. As total protein is specified at 20%, more than one half of the protein consists of what is called the nonessential amino acids. They, as a group, are nutritionally important and provide the animal with the nitrogen needed for protein and tissue synthesis. However, the specific amino acids which may serve for this purpose, as well as the minimum amounts that must be supplied, have not been determined. A 16% protein diet, balanced in respect to methionine and lysine, may be sufficient (*10*). However, the 20% protein level suggested by the National Research Council will be adhered to in this presentation.

Although the National Research Council has stipulated the exact amounts of protein and of essential amino acids needed by the growing chick, a diet conforming to these specifications has never been compounded because it is difficult, if not impossible, to devise such a diet from natural components. Moreover, it is apparently impossible to grow chicks on a diet made up from synthetic amino acids (*18*).

Protein of Mixed Feeds

As the protein of mixed feeds is not balanced, the extent of the deviation

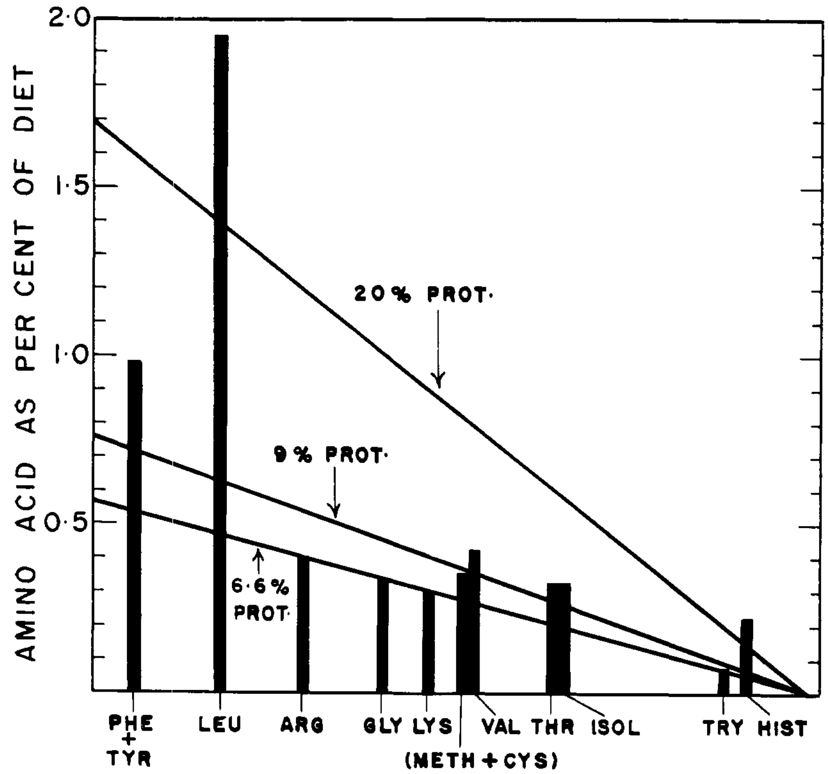

Figure 2. Pattern of essential amino acids in corn in relation to the requirements of the growing chick

is of interest. Two of the most important feed ingredients are corn and soybean oil meal. Corn contains about 9% protein, not the 20% requirement. The pattern of the essential amino acids of corn is shown in Figure 2. According to Almquist (*2*), the amino acid requirements increase or decrease in direct proportion to the protein level. In Figure 2, the 20% protein line is shown and another line which corresponds to a 9% protein ration is drawn from the intercept. If the amino acids were balanced, each bar would come up to the 9% protein line. Obviously, the amino acids in corn are quite unbalanced as there is a relative abundance of leucine and histidine and a relative deficiency of several others, including lysine. By lowering the amino acid balance line, which represents per cent protein, until it touches the amino acid in shortest supply, corn is shown to contain only 6.6% of balanced or efficient protein. The difference, 2.4%, is wasted protein, as Bird has pointed out (*6*). Corn is used in most feeds primarily as a source of energy rather than of protein, but because of the relatively large amounts of it in mixed feeds, the amount of protein that corn contributes is considerable.

Of the many feed ingredients which can be used as a source of protein, the most commonly used is soybean oil meal, the amino acid pattern of which is shown in Figure 3. This ingredient does not provide an ideal protein, yet most manufactured feeds for poultry and swine are based on corn and soybean oil meal. The pattern of essential amino acids in a hypothetical diet made up of corn and soybean oil meal is shown in Figure 4. About 5% of the ration is needed to supply the bird with necessary vitamins and minerals. The remainder is used to provide 20% protein from a mixture of corn (64.9%) and soybean oil meal (30.1%). The protein in this combination is somewhat better balanced than that in either ingredient alone and is short only in one amino acid, methionine. But because of this shortage, the amount of effective protein is only 16.7%, which means that 3.3% protein cannot be used for tissue synthesis and repair. This ineffectual protein can be made useful by supplementing the diet with methionine, as is now being done in the feed industry.

Limiting Amino Acids

Examination of the amino acid pattern of a feed protein is most useful for an appraisal of the extent of the deficiency of the various limiting amino acids. The pattern of the corn-soybean oil meal ration in Figure 4 reveals that methionine is the first and tryptophan the second limiting amino acid. Glycine is drawn as the third, although, according to direct analysis of the corn and soybean oil meal, glycine would be in second place. No beneficial effect on the performance of chicks, from supplementary glycine on a corn-soybean diet, was noted by Machlin, Denton, and Bird (*11*) and by Snyder, Denton, and Scott (*19*); therefore, the glycine values determined by analyses are too low, or as appears more probable, the requirement as stated by the National Research Council is too high.

The corn-soybean oil meal ration of Figure 4 contains tryptophan in the amount specified by the National Research Council, thus permitting the effective protein to be made up to 20% by supplementation with methionine. According to the scale in Figure 4, about 0.1% of methionine would be required. Confirmation that this is the maximum amount which would elicit a beneficial response in chicks may be obtained from published data (*15*). The corn-soybean diet used contained 3% each of alfalfa meal and condensed fish solubles —additions which, by calculation, should not change the amount of methionine needed. Best response was obtained when the diet was supplemented with 0.1% of methionine.

Supplementation of a diet with the first limiting amino acid should be carried out at a level which brings the limiting amino acid into balance with the second limiting amino acid. Supplementation with larger amounts cannot be expected to produce better performance. The amino acid which formerly was second limiting is now first and governs the extent of protein utilization. The biological response to excessive supplementation is often not in accordance with the expected benefit, and even a slight imbalance caused by excessive supplementation with single essential amino acids may be harmful, especially when the protein level is low or when the diet is well balanced in all other respects. Conclusions drawn from such experiments are likely to be erroneous if under the conditions of the experiment, a balance of the first two limiting amino acids was not accomplished.

The corn-soybean oil meal diet of Figure 4 contains 20% protein. What happens to the amino acid pattern when the protein level is changed, by appropriately adjusting the two sources of protein is an important problem as there are feeds for domestic animals ranging from 10% protein concentration for the fattening pig to 30% protein for the turkey poult. In Figure 5, per cent protein in the diet is plotted against per cent of amino acid in protein for lysine, methionine, and the combination of methionine and cystine. As the level of protein is increased by the substitution of corn with soybean oil meal, the relative amount of lysine in the diet increases, while the relative amount of methionine and methionine-plus-cystine decreases. This phenomenon occurs because corn protein is high in methionine and low in lysine, while soybean oil meal protein is low in methionine and high in lysine.

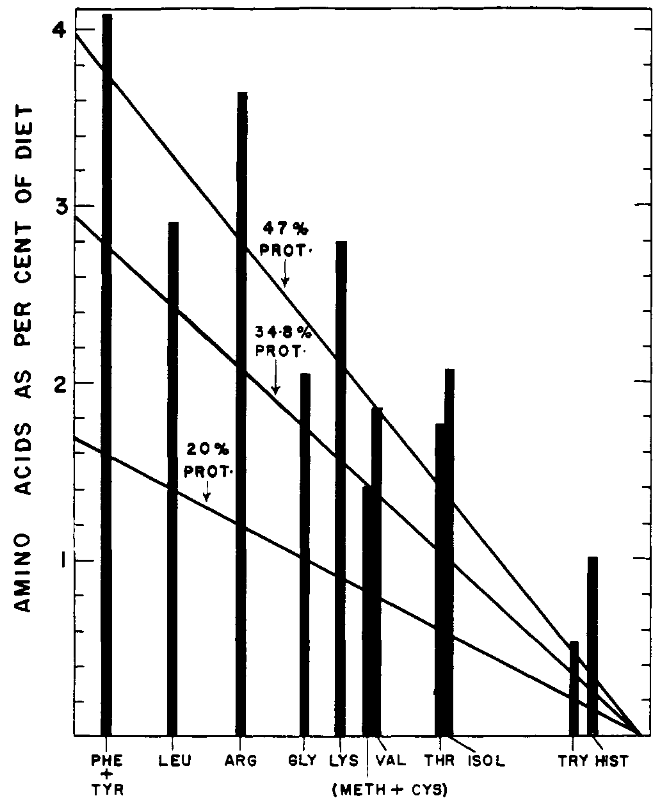

Figure 3. Pattern of essential amino acids in soybean oil meal in relation to the requirements of the growing chick

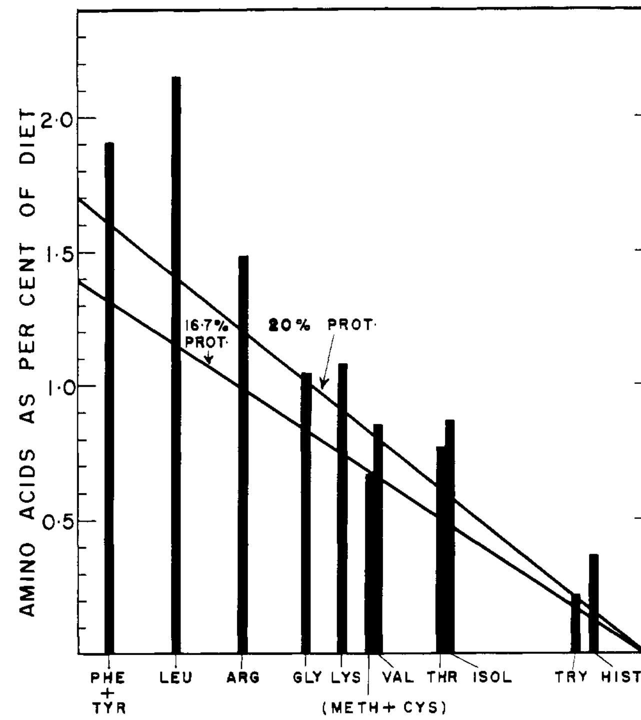

Figure 4. Pattern of essential amino acids in 20% protein corn-soybean oil meal diet in relation to the requirements of the growing chick

Therefore, as the protein level increases, methionine deficiency is likely to occur or to increase. Conversely, as the protein level decreases, lysine deficiency is likely to occur.

Energy Content of Diet

The amino acid requirements for animals are commonly expressed as per cent of diet; however, these requirements can no longer be considered as fixed values. Differences due to species, sex, or age of the animal have long been recognized and investigated; however, the effect which the energy content of the diet has on nutrient requirements was overlooked, although Mendel (*12*) was aware of this consideration 35 years ago.

The most obvious effect of a change in the energy content of a diet is on the efficiency of feed utilization. As the caloric content of a ration is raised—e.g., by the addition of fat—increased feed efficiency results. This effect, as it applies to broilers, is illustrated in Figure 6, in which the energy content of the diet is plotted against the gain over feed ratio. In this graph, as well as in the following discussion, "productive energy" values are used, exclusively, which represent the calories available for useful work, maintenance, and production (*9*). While the position of the line in Figure 6 depends on various factors such as

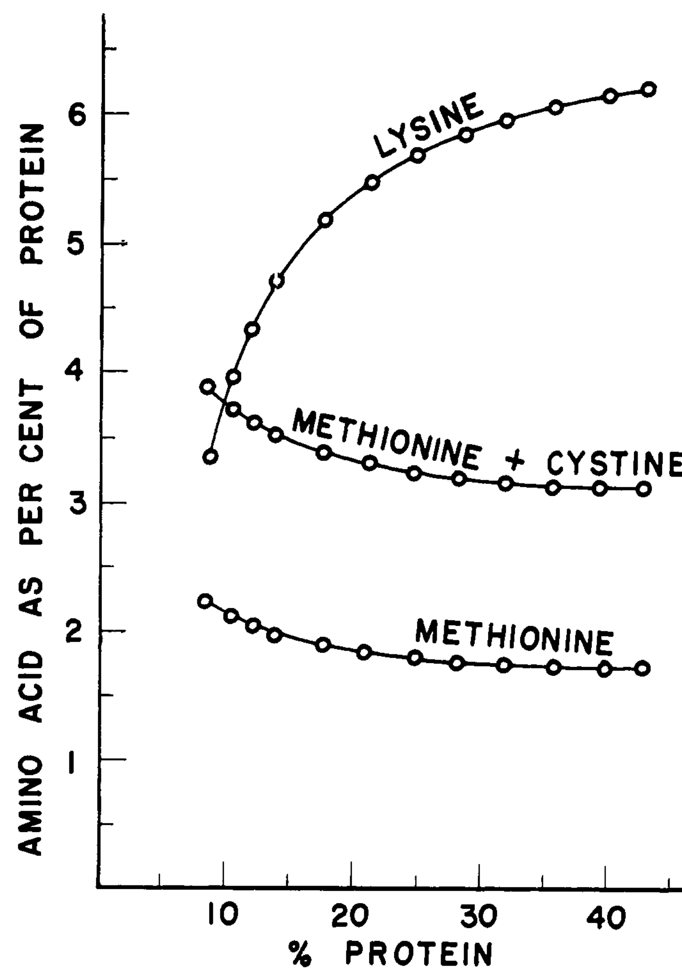

Figure 5. Change in relative amounts of lysine, methionine, and methionine plus cystine in corn-soybean oil meal diets as function of protein content of diet

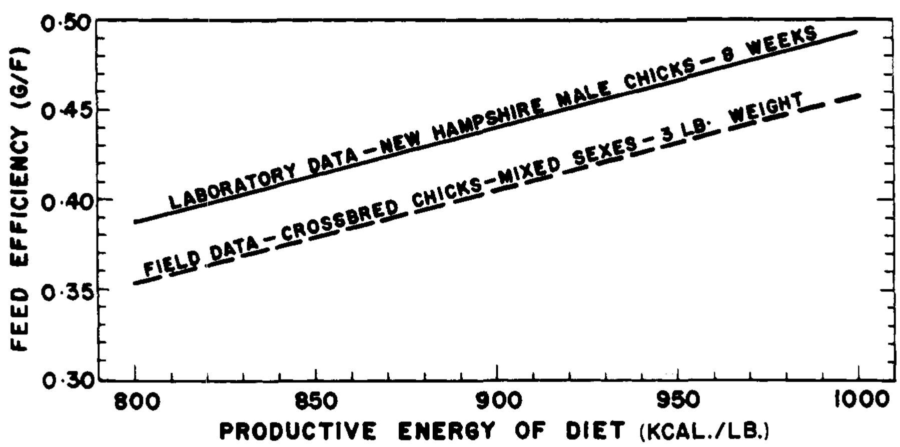

Figure 6. Relationship of energy content of diet to feed efficiency of the chick

strain, sex, and age of the birds, the slope is considered a good approximation of the feed efficiency–energy relationship, provided that nutritionally adequate diets are used.

Animals eat primarily to satisfy their energy requirements and, according to the above discussion, the more energy contained per unit of feed the lower will be the consumption per unit of gain. Yet this smaller amount of feed must contain all essential nutrients in adequate amounts if optimum nutrition is to be obtained. The significance of this consideration, however, had been overlooked until 1955, when energy was shown to be one of the controlling factors in amino acid requirements (*4*).

The recognition of this relationship stemmed from a careful study of the methionine requirement of the growing chick on many diets of widely divergent energy content and was confirmed by specially designed experiments. Using isonitrogenous diets containing 20 to 22% of protein and varying the energy content over a 200-kcal. per pound range, the methionine requirement for the growing chick was shown to be related to the dietary energy content. A corn-soybean ration containing peas was used. The amino acid pattern, shown in Figure 7, demonstrates the deficiency of methionine. The energy content was varied by changing the amounts of fiber and fat in the diet. After adding graded amounts of methionine to these diets, the responses in growth and feed efficiency of several groups of chicks were observed. These results (Figure 8) show the responses to the three diets of approximately 800, 900, and 1000 kcal. of productive energy per pound. The methionine content of the diets is plotted against the index of performance, a value which combines the observed data for growth and feed efficiency. (The index of performance is obtained by multiplying the weight gains by the gain per feed ratio.)

As seen in Figure 8, optimum performance is achieved at different levels of methionine for diets of different caloric content. Results from a variety of experiments show that the methionine requirement may be expressed as a straight-line function of the energy content of the diet according to the formula $Y = 0.000736\,X - 0.2269$, in which Y represents per cent methionine in the diet and X the productive energy content per pound of diet. This means that the methionine requirement, expressed as per cent of diet, is not constant but increases rather steeply with increasing energy content of the diet. The same principle is believed to apply to all essential amino acids. This has been proved experimentally in a number of instances: for the lysine requirement of the growing rat (*16*), for the chick (*20*) and for the methionine requirement of the turkey (*3*).

With this principle firmly established, its limitations might be considered briefly. When an animal is not able to

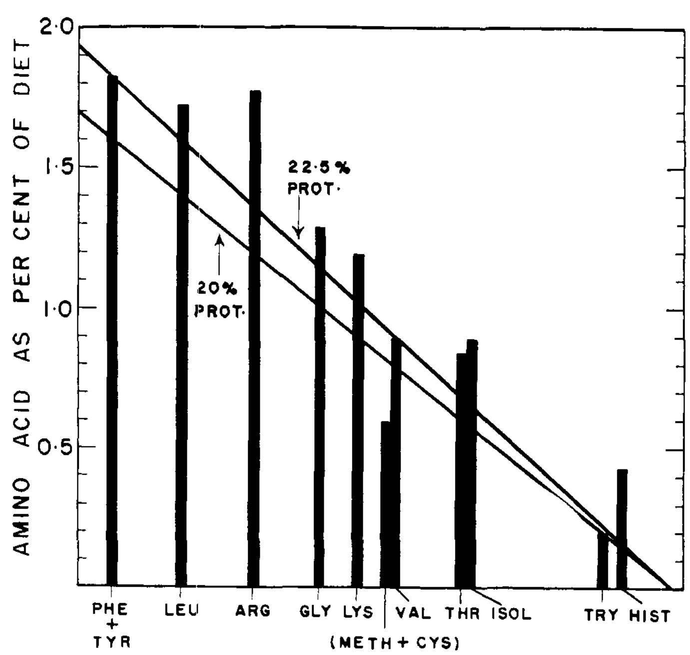

Figure 7. Pattern of essential amino acids in experimental ration in relation to the requirements of the growing chick

(Used in proving the methionine-energy relationship)

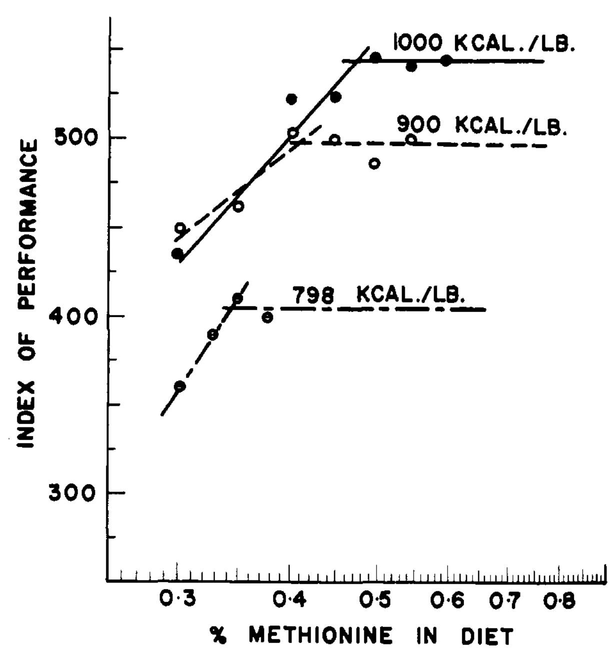

Figure 8. Methionine requirement of the growing chick consuming 21% protein diets of different energy content

consume enough to satisfy its energy requirements because of the ration's low caloric content, the principle obviously does not apply. With increasing energy content, limitation occurs when one or more of the other nutrients becomes limiting. For example, the amino acid pattern of the diet shown in Figure 7 suggests that after 0.2% methionine addition to the diet further supplementation with methionine should not improve the diet, as methionine is no longer the only limiting amino acid.

In the experiments shown in Figure 8, maximum performance at 1000 kcal. was obtained when 0.5% total, including 0.2% added, methionine was in the diet. If the energy content were increased even further—e.g., to 1200 kcal.—best performance should still be obtained with 0.5% total methionine in the diet. A 4-week battery test has shown this assumption to be correct. The same diet was used as in the earlier published account (*4*) but the amount of corn was decreased to 13.5%, while the amount of soybean meal was increased to 19.5% of the ration. Larger amounts of fat were added in order to obtain diets with higher energy content. These changes slightly decreased the methionine present so that there was now, by calculation, a deficiency of 0.23% of methionine. The results of this experiment are shown in Table I and indicate that, as predicted, no increase in methionine requirement was observed when the energy content of the diet was increased from 1000 to 1200 kcal.

Table I. Methionine Supplementation of Diets Adequately Balanced with Respect to Energy and Second Limiting Amino Acid

	Av. Wt. Gain, G.	Feed Consumed/Gain	Index of Performance
Basal, 20% protein, 1000 kcal.	355	2.36	150
Basal + 0.20% DL-methionine	436	1.96	222
Basal + 0.25% DL-methionine	452	1.93	234
Basal + 0.30% DL-methionine	453	1.94	234
Basal, 20% protein, 1200 kcal.	262	2.40	109
Basal + 0.20% DL-methionine	404	1.86	217
Basal + 0.25% DL-methionine	410	1.82	225
Basal + 0.30% DL-methionine	405	1.86	218

Effect of Different Protein Levels

Following this work on isonitrogenous diets, the effect of a change in protein level on the amino acid–energy relationship was studied. A change of protein level usually brings about not only a change in the ratio of the amino acids in the diet, but also a change in energy content. Low protein diets contain a greater amount of corn, which is a high energy feedstuff, than high protein diets containing a relatively greater amount of soybean oil meal which is a low energy feedstuff. In order to maintain energy level or to raise it, when the protein level is increased, some of the carbohydrate or fiber in the diet must be replaced by fat. Studies with diets of different protein content at several energy levels show that the methionine requirement of the chick is dependent upon both the energy and the protein level (*14*).

According to earlier experimental evidence (*2*) with increasing protein levels, the requirements for the individual amino acids, expressed as per cent of diet, increased proportionately. This principle has now been confirmed, but it was found to apply only when the diet contained sufficient energy to permit the animal to make full use of the protein for growth. If, however, insufficient energy is available from the nonprotein sources, apparently the animal will use some of the protein to satisfy its energy requirements. Under such conditions, the noneffective part of the protein, in preference to the balanced portion, may be used for energy. This interpretation is based on the finding that, with restricted energy content, the requirement for methionine did not increase in proportion to the increase in protein. More work is needed to show the quantitative interrelationships among protein level, energy level, and level of the limiting amino acid.

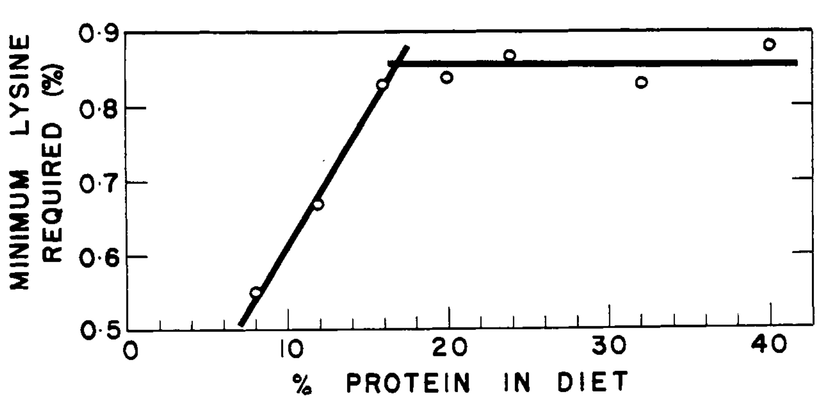

Figure 9. Lysine requirement of the growing rat consuming diets of different protein content

These observations permit a new interpretation of recent data (Figure 9), presented by Bressani and Mertz (*7*). Using isocaloric diets, they found that, upon increasing protein content, higher levels of lysine were needed by the growing rat, until at about 16% protein, 0.83% lysine was required. Further increases in protein level did not increase the lysine requirement, showing that, with 16% protein, balance was reached between the protein and the energy content of the diet. Upon further increases in protein content the amount of calories from nonprotein sources was decreased, thus forcing the animal to use protein as a source of energy rather than as a source of nitrogen.

That this interpretation may be correct is borne out by an experiment in which the lysine requirement of rats was determined on the 24% protein diet of Bressani and Mertz. When the energy content was increased from 700 to 1500 kcal. per pound at intervals of 200 kcal., the lysine requirement was found to increase steadily as shown in Figure 10.

Discussion

These new developments and concepts of amino acid utilization should have a considerable impact on the manner in which amino acid requirements will be stated in the future. They should not be quoted as fixed values when expressed as per cent of diet unless the energy level is also stated. Ideally, the relative requirements of the essential amino acids would be found to remain constant regardless of energy or protein level of the diet (*7*). However, a reappraisal of the requirements of each of the essential amino acids is necessary as there is reasonable doubt as to the accuracy of several of these values.

Either methionine or lysine is, as far as is known, the first limiting amino acid in most mixed feeds for domestic animals. Hence, the feed industry is making extensive use of the availability of synthetic methionine. According to compilations from the Tariff Commission, methionine production in the United States in 1954 was about a million pounds and considerably larger amounts are being manufactured now. According to calculations carried out by Bird (6), the cost of methionine when added to a methionine-deficient diet is about equal to the value of the salvaged protein and energy. As the result of correcting a methionine deficiency, the broiler grower will experience improved performance of his flock and the profit obtained from the added methionine will be well in excess of its cost. Many laboratory tests, field trials, and commercial broiler operations have confirmed this conclusion.

Lysine is produced in limited quantities in the form of the naturally occurring optical isomer and is sold as the monohydrochloride. Production has not as yet reached a volume sufficiently large to permit sale at a price which would make lysine an attractive ingredient for animal feeds, even though there are so many protein sources which are decidedly deficient in lysine. The considerations which have compelled the feed manufacturer to demand greater efficiency for his feeds and to put into effect a program of improving the protein by amino acid supplementation may ultimately apply to human foods. Lysine has already found its way into certain specialty breads and this trend is increasing and is expected to result in general nutritional benefits to people of all age groups.

The 16% protein ration suggested for pigs up to 70 pounds live weight should contain 1.0% lysine according to the National Research Council recommendations (13). Manufactured rations for the weanling pig are borderline in respect to lysine content, and although evidence is now accumulating that the recommended figure may be too high, the rations nevertheless appear to be marginal in lysine. Experiments with weanling rats have shown that the rate of growth is easily depressed when a lysine deficiency exists. Within certain limits, rate of growth was found to be directly proportional to lysine intake, as seen from Table II (17). Such studies with rats can be useful as a basis for later experiments with pigs.

The feeding of this country's ever-increasing population is a difficult problem, but research scientists will learn how to produce more and how to use what is available more economically. Methionine and lysine and perhaps other essential amino acids, obtainable at reasonable costs, will permit the saving of vast amounts of protein through proper applications of the principles which have been discussed.

Commercial turkey starter rations now contain 28 to 32% protein. The need for this high protein level is attributed, largely, to a specifically high requirement for lysine and methionine. This amount of protein can safely be reduced by almost one third (5). In the experiment shown in Table III, the 20% protein diet did not perform as well as the same diet containing 28% protein. When both were appropriately supplemented, the low protein diet with methionine and lysine and the high protein diet with methionine, the two diets performed equally well.

A 16 to 18% protein pig starter diet was effectively reduced to a 12 to 14% protein level (8). Earlier, reference was made to a broiler diet containing only 16% protein which performed as well as one containing the usual 20% protein (10). All of this has been accomplished by using conventional feed ingredients. In addition, many sources of protein which are not used now could be used if the need arises. Methionine has taken a place alongside the vitamins as a regular feed ingredient and lysine may soon follow into the same position.

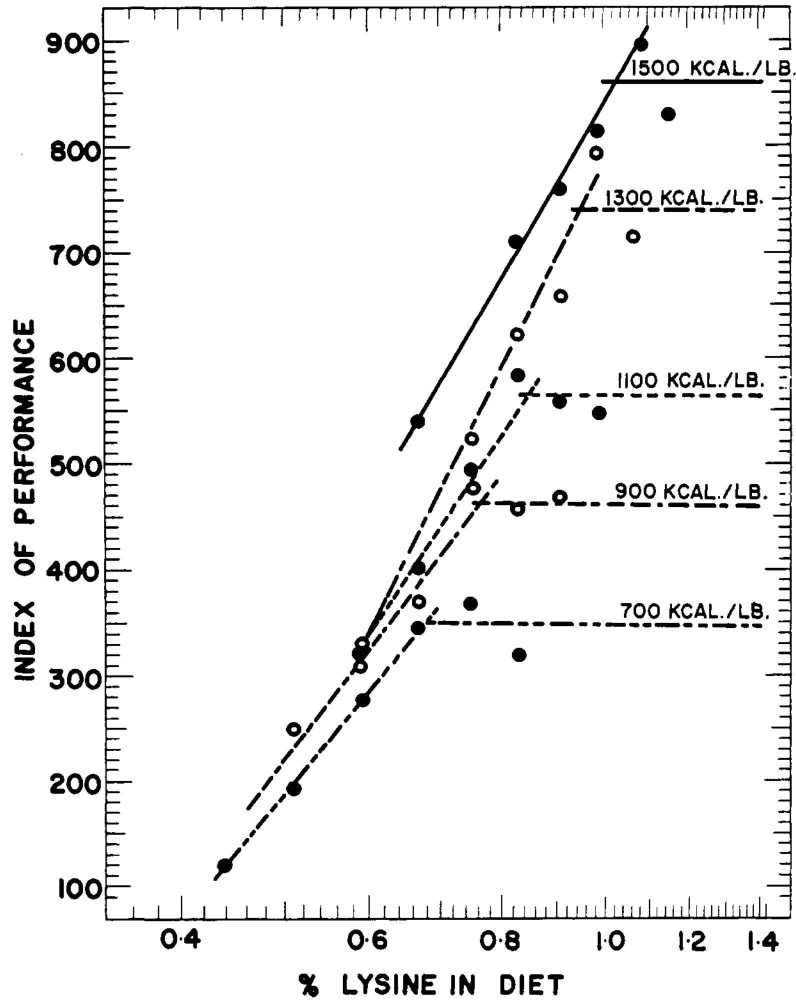

Figure 10. Lysine requirement of the growing rat consuming 24% protein diets of different energy content

Table II. Growth Response of Weanling Rats to Increasing Levels of Lysine

(5-Week Data)

Total Lysine in Diet, %	*Av. Wt. Gain, G.*	*Gain/Feed Consumed*	*Feed Consumed, G.*	*Mg. Lysine/G. of Gain*
0.29	58	0.13	445	22.3
0.39	102	0.19	547	20.5
0.48	135	0.24	570	19.9
0.67	180	0.28	642	23.8

Table III. Growth and Feed Efficiency of Jersey Buff Poults on 20% and 28% Protein Diets

Treatment	*6-Week Gain, G.*	*Feed Gain*
20% protein	671	2.30
20% protein + methionine + lysine	807	2.17
28% protein	764	2.32
28% protein + methionine	812	2.27

Directly or indirectly, these two essential amino acids will contribute to the welfare of mankind.

Literature Cited

(1) Almquist, H. J., *Arch. Biochem. Biophys.* **48,** 482 (1954).
(2) Almquist, H. J., *Proc. Soc. Exptl. Biol. Med.* **72,** 179 (1949).
(3) Baldini, J. T., Marvel, J. P., Rosenberg, H. R., Informal Poultry Conference, Federation Meetings, Chicago, Ill., 1957.
(4) Baldini, J. T., Rosenberg, H. R., *Poultry Sci.* **34,** 1301 (1955).
(5) Baldini, J. T., Rosenberg, H. R., Waddell, J., *Ibid.*, **33,** 539 (1954).
(6) Bird, H. R., *Feed Age* **6** (No. 11), 34 (1956).
(7) Bressani, R., Mertz, E. T., *Federation Proc.* **15,** 544 (1956).
(8) Catron, D. V., Jensen, A. H., Homeyer, P. G., Maddock, H. M., Ashton, G. C., *J. Animal Sci.* **11,** 221 (1952).
(9) Fraps, G. S., *Texas Agr. Expt. Sta. Bull.* **678,** 1946.
(10) Johnson, J. E., *Feed Bag* **29** (No. 1), 26 (1953).
(11) Machlin, L. J., Denton, C. A., Bird, H. R., *Poultry Sci.* **31,** 110 (1952).
(12) Mendel, L. B., "Nutrition: The Chemistry of Life," p. 126, Yale Univ. Press, New Haven, Conn., 1923.
(13) National Research Council Publ. **301,** "Nutrient Requirements for Poultry," rev. 1954; Publ. **295,** "Nutrient Requirements for Swine," rev. 1953; "Recommended Nutrient Allowances for Dairy Cattle," rev. 1950; "Recommended Nutrient Allowances for Beef Cattle," rev. 1950; "Recommended Nutrient Allowances for Sheep," rev. 1949; "Recommended Nutrient Allowances for Horses," 1949; "Nutrient Requirements for Foxes and Mink," 1953; Publ. **300,** "Nutrient Requirements for Dogs," 1953; and Publ. **302,** "Recommended Dietary Allowances," rev. 1953.
(14) Rosenberg, H. R., Baldini, J. T., *Poultry Sci.* **35,** 1168 (1956).
(15) Rosenberg, H. R., Baldini, J. T., Sunde, M. L., Bird, H. R., Runnels, T. D., *Ibid.*, **34,** 1308 (1955).
(16) Rosenberg, H. R., Culik, R., *J. Animal Sci.* **14,** 1221 (1955).
(17) Rosenberg, H. R., Rohdenburg, E. L., *Arch. Biochem. Biophys.* **37,** 461 (1952).
(18) Scott, H. M., Abstract of Papers, Animal Nutrition Research Council Meeting, October 17, 1956.
(19) Snyder, J. M., Denton, C. A., Scott, H. M., *Poultry Sci.* **35,** 852 (1956).
(20) Williams, M. A., Grau, C. R., *J. Nutrition* **59,** 243 (1956).

Received for review February 4, 1957. Accepted May 6, 1957. Adapted from an address delivered at the Division of Chemical Marketing and Economics, 130th Meeting, ACS, Atlantic City, N. J., September 1956.

IRRADIATION EFFECTS IN MEAT

Production of Carbonyl Compounds during Irradiation of Meat and Meat Fats

O. F. BATZER, M. SRIBNEY, D. M. DOTY, and B. S. SCHWEIGERT

American Meat Institute Foundation and Department of Biochemistry, University of Chicago, Chicago, Ill.

Investigation of the chemical changes that occur when meat and meat products are subjected to gamma radiation for sterilization indicated the formation of carbonyl compounds. Extraction procedures and the behavior of the compounds with various chromatographic solvent systems suggested that those obtained from irradiated meat differ from those obtained from irradiated fat. Carbonyl compounds increase in both meat and fat with increasing irradiation dosages.

PREVIOUS STUDIES in this laboratory on irradiated meat related to changes that occur in the meat pigment myoglobin (*4, 5*), sulfur-containing constituents (*1, 7*), proteolytic enzyme activity (*2*), and peroxide and free fatty acid production in meat fats (*10*). In the latter paper, reference was made to preliminary experiments which showed that an increased amount of carbonyl compounds was produced during irradiation of meat fat at 2×10^6 rep of gamma ray treatment. These changes have also been observed in model systems. Mead and associates (*8, 9*) stated that irradiation causes induction of autoxidation of methyl linoleate, and Dugan and Landis (*3*) reported an increase in peroxides and carbonyl compounds from the irradiation of methyl oleate.

Although the products from the fat in irradiated meat do not contribute directly to the off-odors, meats with a high fat content do not develop off-odors to the same extent as do leaner meats. The possibility of carbonyls from fat being involved in the suppression of off-odors merited further investigation of these compounds.

In this study, experiments have been extended to determinations of carbonyl compounds extracted with benzene from beef and pork fats, and those extracted by acidic solutions and/or benzene from ground beef muscle irradiated under various conditions and dosages.

The results indicate that carbonyl compounds produced in irradiated ground beef muscle differ from those obtained from irradiated beef or pork fat. The amounts of the carbonyl compounds were shown to increase with the use of increasing irradiation dosage levels.

Experimental

The benzene and alcohol used in these experiments were carbonyl free. The 2,4-dinitrophenylhydrazine was twice recrystallized from carbonyl-free methanol.

Benzene Extraction. A 10-gram sample of irradiated meat or fat, mixed in a Waring Blendor for 2 minutes with 90 ml. of benzene, was filtered and washed with sufficient benzene to make 100 ml. of the mixture. Then 2-ml. aliquots were transferred to a 25-ml. volumetric flask and 3 ml. of 4% trichloroacetic acid in benzene and 3 ml. of a saturated benzene solution of 2,4-dinitrophenylhydrazine were added. This mixture was maintained at 60° C. for 30 minutes in a water bath. After cooling to room temperature, 5 ml. of 4% potassium hydroxide in ethyl alcohol was added and the solution was brought to volume with ethyl alcohol. The absorbance values were determined with a Beckman spectrophotometer at 430 mμ. The 2,4-dinitrophenylhydrazone of *n*-heptaldehyde in appropriate concentrations was used for the standard curve.