



AMINO-ACIDS ESSENTIAL FOR THE GROWTH OF YOUNG GREEN SEA TURTLES (*Chelonia mydas*)

James R. Wood, Jr. Department of Biological Sciences University of Arizona Tucson, Arizona 85721

Proceedings of the World Mariculture Society 5: 233-248

ABSTRACT

INTRODUCTION

DETERMINATION OF AMINO ACID REQUIREMENTS

DISCUSSION OF RESULTS

ACKNOWLEDGEMENTS

LITERATURE CITED

ABSTRACT

The development of a completely defined amino acid test diet suitable for hatchling sea turtles is briefly discussed. The results of a series of experiments designed to determine the nutritionally essential amino acids required by the hatchling green sea turtle, *Chelonia mydas*, are presented. Of the 18 amino acids of nutritional significance, nine are shown to be clearly essential, eight are shown to be clearly non-essential, and one amino acid is shown to be possibly semi-essential. Possible relationships of the reported results to the protein and amino acid requirements of turtle age classes other than hatchlings are mentioned. Directions of future research and implications for the development of commercial turtle feeds are suggested.

INTRODUCTION

Of the many amino acids which are constituents of animal protein and which must be available to the animal's cells if protein is to be produced, 18 occur most commonly and are considered to be nutritionally significant. Amino acids which the animal can synthesize are deemed nutritionally non-essential while those which must be provided by the diet are designated as nutritionally essential. The qualitative and quantitative amino acid requirements of various mammals, birds, fish, and invertebrates have been determined during the last 25 years. Information concerning the nutritional requirements of amphibians and reptiles is almost non-existent except for general information on natural foods on which these animals can be maintained in captivity.

The first dietary study on turtles was conducted by Pearse, et. al. (1925). Three species, a painted turtle, a gopher tortoise, and a terrapin were fed different rations of "pure" foods such as sand, casein, eggs, lettuce, meal worms, dextrin, wheat, and cod-liver oil. The authors concluded that the food requirements of chelonians as poikilothermal animals are similar to those of homoiothermal animals.

Coulson and Hernandez (1964), in discussing results of blood amino acid studies on the alligator, suggest that the essential amino acids required by the alligator are the same as those required by mammals with the exception of lysine, for which they cite evidence of synthesis of this normally essential amino acid from arginine and citrulline in the alligator. The alligator and turtle, *Pseudomys* (sic.) *scripta elegans* in general metabolize the "essential" amino acids more slowly than the "non-essential" amino acids and lysine is tentatively identified as a conversion product of arginine in the turtle and alligator (Coulson and Hernandez, 1965).

Work on determination of the nutritionally essential amino acids required by the hatchling green sea turtle, *Chelonia mydas*, was initiated for two reasons:

- 1) On a pure science level, it would be the first time that the qualitative amino acid requirements of a reptile had been determined.
- 2) On an applied level, information concerning the nutritional requirements of the species in its infancy as a "domestic" and cultured animal could be very important in the development of economical rations.

DETERMINATION OF AMINO ACID REQUIREMENTS

Approaches to the Problem

There are two common approaches to the problem of determining the nutritionally essential amino acids required by an animal. Borman, et al. (1946) and Womack and Rose (1947) introduced the use of a synthetic diet made up of purified substances to determine amino acid requirements in their studies on the rat. This method involves the planned deletion of selected test amino acids from the prepared diet. If growth is halted or severely depressed, the deleted amino acid is considered to be essential. The second approach utilizes the injection of a radioactive carbon source, usually labelled glucose. After a period of time, tissue samples are removed and the constituent amino acids are analyzed for radioactive carbon. Heavily-labeled amino acids will have been synthesized by the animal from the carbon source and are considered non-essential, while non-labeled amino acids are considered to be nutritional essentials.

The purified synthetic diet method was chosen for the present study on sea turtles, since once the purified diet was developed it could be used to determine quantitative as well as qualitative amino acid requirements.

Development of a Purified Diet

A purified diet for the determination of nutritionally essential amino acid requirements must be completely defined chemically; it must allow the manipulation of any one or more amino acids and, if growth is to be the test criterion; it must maintain the physical condition and promote growth of the test animal.

The development of amino acid test diet for young green sea turtles has been complicated by two factors:

- 1) Hatchlings can be obtained on the average of only twice a year (Northern hemisphere and Southern hemisphere breeding seasons),
- 2) The cost of purified amino acids is high.

For these reasons, it was often impossible in the work reported here to repeat preliminary experiments and it was some times necessary to accept and act upon results not substantiated as fully as would have been desired under other circumstances.

Preliminary experiments during the development of the purified diet were conducted in a wide variety of water systems, always utilizing either artificial or natural sea water at a water temperature between 24°C and 29°C. Hatchlings were fed all they would eat three to four times a day, 6 days a week. Individual weights were determined each week following the day of fasting. The initial purified diet was based on the composition of shrimp as given by the United States Department of Agriculture (1963) since both Harrison (1955) and Caldwell (1962) had reported raising hatchlings for up to three years on a diet of shrimp. The diet contained (wet weight) 18.5% amino acid mixture, 1.5% dextrose, 2.5% vitamin mixture, 1.4% salt mixture, 2.0% corn oil, 3.0% agar as a binder, and 71.1% distilled water. The amino acid mixture contained the 18 common amino acids in the proportion they occur in shrimp as reported by Borgstrom (1962).

The original test diet evolved by trial and error experimentation into the test diet which is currently used.

Some steps in this process were as follows:

- 1) The amount of carbohydrate (dextrose) was increased from 1.5% to 20.0% to provide energy;

2) Potato starch was substituted for dextrose as the carbohydrate material when it appeared that a larger molecular size was desirable (less active leaching before ingestion, apparent improved growth rates);

3) The salt mixture was supplemented with additional sodium and potassium to more closely approximate the mineral composition of shrimp;

4) Substitution of carboxymethyl cellulose for agar as the binder when it was discovered that this reduced obstructions in the large intestine; 5) Use of diammonium citrate to replace the nitrogen of deleted amino acids;

6) Change from the amino acid pattern of shrimp to that of casein when it was found that this produced better growth rates;

7) Doubling of protein (amino acid) complement from 18% to 36% wet weight of the diet and halving of starch complement from 20% to 10% wet weight, since this resulted in improved rates of growth;

8) Deletion of L-alanine from all diets after tests indicated that this amino acid was nonessential and consistently satisfactory results were obtained without it;

9) Substitution of 'Alphacel'" (ground cellulose) for deleted amino acids rather than using diammonium citrate after learning that diammonium citrate had been shown to have a negative effect on chinook salmon (DeLong et al., 1959).

The composition of the amino acid test diet as it is currently used is given in **Table 1**.

Table I - Composition of amino acid test diet

Ingredient	gm/100 gm of diet *(1)
L Lysine - HCL	1.44
L Histidine -HCL- H ₂ O	1.04
L Arginine HCL	1.34
L Aspartic acid	2.64
L Threonine	1.46
L Serine	1.98
L Glutamic acid	8.28
L Proline	3.70
Glycine	1.28
L Cystine	0.28
L Valine	2.02
L Methionine	0.70
L Isoleucine	1.52
L Leucine	3.26
L Tyrosine	1.96
L Phenylalanine	1.70
L Tryptophan	0.36
Total	34.96

Potato starch	10.00
Vitamin diet fortification mixture	2.50
Hawk oser salt mixture No. 3	1.40
Potassium phosphate monobasic	0.40
Sodium chloride	0.20
Corn oil	2.00
Carboxymethyl cellulose sodium	5.00
Distilled water	43.54

*(1) in deleted diets the deleted amino acid was replaced by an equal weight of "ALPHACEL", a ground cellulose obtained from Nutritional Biochemical Co.

Preparation and Storage of the Diet

The amino acid mixture, potato starch, vitamin mix, salt mix, and corn oil were thoroughly mixed. Distilled water heated to 85-90°C. was added to the above ingredients and stirred until soluble components were dissolved. The carboxymethyl cellulose was then blended into the diet until a homogeneous mixture of dough-like consistency was produced. The diets were then stored in widemouthed jars at a temperature of approximately 10°C., until used.

Determination of 6 Non-essential Amino Acids

In an experiment conducted during the latter stages of the development of the purified diet, 19 groups of six hatchlings each were fed synthetic diets. The control group received a diet containing all 18 amino acids, while each of the remaining 18 groups received variations of the diet, each missing a different amino acid. In each case the amount of nitrogen removed from the diet by deleting an amino acid was replaced by adding the appropriate amount of diammonium citrate. The composition of this diet is given by [Table 2](#).

Table 2.- Composition of experimental diet including L Alanine and diammonium citrate.

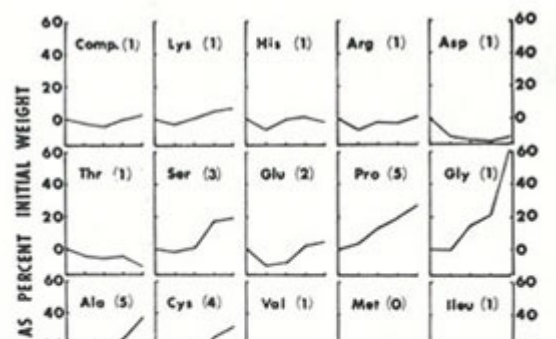
Ingredient	gm/100 gm of diet *(1)
L Lysine . HCL	1.44 (1.78)
L Histidine HCL H ₂ O	1.04 (1.68)
L Arginine HCL	1.34 (2.88)

L Aspartic acid	2.64 (2.24)
L Threonine	1.46 (1.38)
L Serine	1.98 (2.12)
L Glutamic acid	8.28 (6.34)
L Proline	3.70 (3.63)
Glycine	1.28 (1.94)
L Cystine	0.28 (0.26)
L Valine	2.02 (1.96)
L Methionine	0.70 (0.32)
L Isoleucine	1.52 (1.30)
L Leucine	3.26 (2.82)
L Tyrosine	1.96 (1.22)
L Phenylalanine	1.70 (1.16)
L Tryptophan	0.36 (0.36)
Total	34.96
Potato starch	10.00
Vitamin diet fortification mixture	2.50
Hawk oser salt mixture No. 3	1.40
Potassium phosphate monobasic	0.40
Sodium chloride	0.20
Corn oil	2.00
Carboxymethyl cellulose sodium	5.00
Distilled water	42.50

(1) indicates the amount of diammonium citrate used to replace nitrogen of deleted amino acid. The amount of water varied depending upon the amount of diammonium citrate used.

FIGURE 1. Rates Of Growth Of Hatchling Green Sea Turtles Fed Synthetic Diets. () Indicates Number Of Hatchlings Surviving Experimental Period.

During the fourth week of this experiment a severe disease problem developed which resulted in a



68% mortality and the virtual elimination of several of the groups, including the control. Since disease was so obviously a major factor in this experiment, no firm conclusions could be reached concerning the essential nature of the deleted amino acids. Six test groups did, however, gain relatively high percentages of their initial weights and generally had a lower mortality than did the other 13 groups. **Figure 1** shows the rate of growth and number of surviving turtles in each group. The above six groups were on diets lacking either alanine, proline, serine, cystine, tyrosine, or glycine, suggesting that these amino acids might be nutritionally non-essential.

To further test if the indicated six amino acids were nonessential, selected survivors of the above experiment were used in a second experiment. All were fed shrimp for 11 days and were then weighed. By the end of this period, losses due to disease had tapered off. The 16 hatchlings which showed the greatest percent gain during the 11 days on the shrimp diet were divided into four groups. During the second test, the turtles were fed during the day in fresh water and were placed in artificial sea water each evening.

Group 1 received the standard control diet as shown in Table 2, with 36% protein complement using diammonium citrate to replace alanine. The other three groups received diets with further deficiencies and with variations in make-up of the total protein complement. The diet for Group 2 contained 18% protein, made up of the 12 amino acids other than those six presumed above to be nonessential, the diet for Group 3 contained the same 12 amino acids, but at double quantities to produce a 36% protein level. The diet for Group 4 contained the 12 amino acids at the same level as for Group 2, but had the presumed non-essential amino acid glycine added to make the total protein up to 36%. The exact make-up of diets 2, 3 and 4 is presented in **Table 3**

Table 3.- Composition of experimental diets

Ingredient	gm/100 go of diet *(1)		
	Group 2 diet	Group 3 diet	Group 4 diet
L Lysine . HCL	1.01	2.02	1.01
L Histidine HCL H ₂ O	0.72	1.44	0.72
L Arginine . HCL	0.94	1.88	0.94
L Aspartic acid	1.84	3.68	1.84
L Threonine	1.03	2.06	1.03
L Glutamic acid	5.78	11.56	5.78
L Valine	1.40	2.80	1.40
L Methionine	0.49	0.98	0.49
L Isoleucine	1.06	2.12	1.06
L Leucine	2.27	4.54	2.27
L Phenylalanine	1.19	2.38	1.19
L Tryptophan	0.25	0.50	0.25

Glycine	-	-	11.40
Total	17.98	35.96	29.38*
Potato starch	28.00	10.00	16.60
Vitamin diet fortification mixture	2.50	2.50	2.50
Hawk oser salt mixture No. 3	1.40	1.04	1.04
Potassium phosphate monobasic	0.40	0.40	0.40
Sodium chloride	0.20	0.20	0.20
Corn oil	2.00	2.00	2.00
Carboxymethyl cellulose sodium	5.00	5.00	5.00
Distilled water	42.52	42.52	42.52

* This total is equivalent to 36 gm of protein due to the rela-high level of nitrogen contained in glycine.

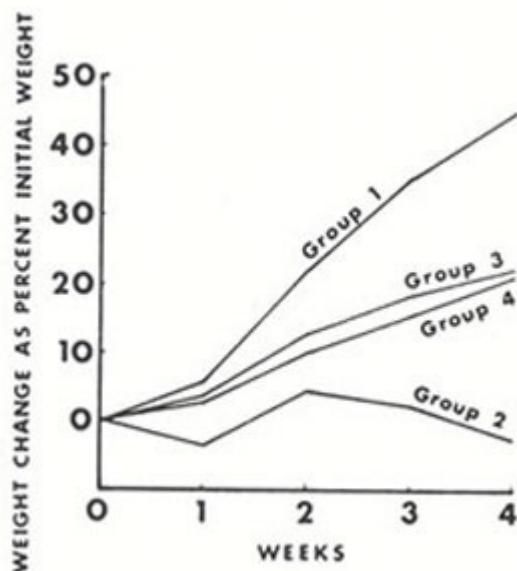


FIGURE 2. Rates Of Growth Of Hatchling Green Sea Turtles Fed 4 Different Synthetic Diets

As shown in **Figure 2**, Group 1 gained the most. Group 2 (low protein level) lost weight, and Groups 3 and 4 gained approximately equal amounts. The excellent growth obtained by the control confirmed alanine as being a non-essential amino acid. The 22.6% weight increase for Group 3 corresponds to an average gain of 16 g per turtle, which supports the conclusion that the deleted six amino acids are non-essential. Growth response of Group 4 indicates that glycine is efficient as a replacement source of nitrogen.

Determination of Essential Amino Acids

Thirteen groups of eight hatchlings each were fed test diets based on the formula given in **Table 1**. The control group received the complete test diet, the other 12 groups each received the diet with a single amino acid deleted (either lysine, histidine, arginine, aspartic acid, threonine, glutamic acid, valine, methionine, isoleucine, leucine, phenylalanine, or tryptophan). The hatchlings were fed during the day in plastic dishpans containing fresh water, then transferred in the evening to similar dishpans containing filtered artificial sea water. Turtles were fed as much as they would eat each day for 6 days a week. Individual weights were determined on the day after fasting. The regime was continued for 3 weeks, then all were fed the control diet during the fourth and final week of the experiment.

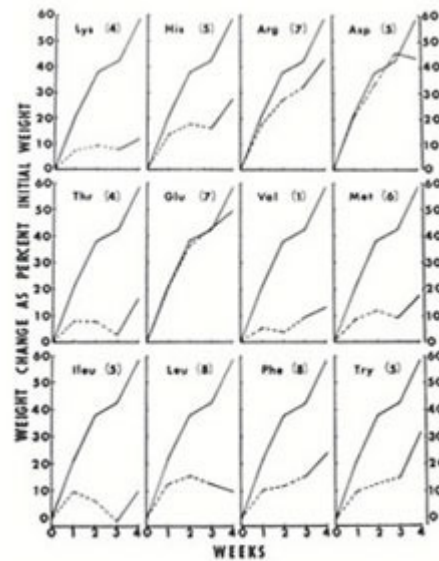
Figure 3 shows the percentage of initial weight gained for each group receiving a deleted diet, compared to percentage of initial weight gained in the control group. It can be seen that only the

groups fed the aspartic acid-free or the glutamic acid-free diets had rates of gain equal to the control. The groups receiving diets lacking either lysine, histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, or tryptophan had rates of growth less than the control but, with the exceptions of the leucine-free and the valine-free diet groups, they responded with an increased rate of growth upon receiving the control diet in the fourth week. The group receiving the arginine-free diet did not gain as well as the control, but it gained better than the other low-growth groups.

During the last 2 weeks of the experiment, a number of turtles died, apparently from disease. Total mortality was 32%. The group fed the valine-free diet had only one turtle remaining at the end of the experiment, while the other groups lost an average of two turtles each.

Duncan 5 multiple-range test on percent of initial weight gain values of each group at the end of the third week indicated no statistically significant difference ($P=.05$) between the control and the glutamic acid-free or aspartic acid-free diet groups. A significant difference did exist between the control and all other groups. The arginine-free diet group was significantly different from all other groups, having gained more than the groups lacking either lysine, threonine, histidine, methionine, valine, isoleucine, leucine, tyrosine, tryptophan, or phenylalanine, and having gained less than either the control, glutamic acid-free, or aspartic acid-free diet groups.

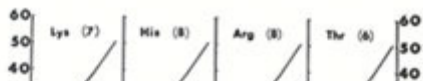
FIGURE 3.
Rates Of Growth Of Hatchling Green Sea Turtles
Fed For 3 Weeks On Synthetic Diets Each Lacking a
Different Amino Acid
 (-----)
Compared To Rate Of Growth Of Hatchlings Fed The
Control Diet (_____)
Number In () Indicates The Number Of Hatchlings
Surviving Experimental Period



Since disease became a problem during the last 2 weeks of the above experiment, it was felt that it would be necessary to retest those amino acids which appeared to be essential. Upon arrival of additional hatchlings the above experiment was repeated with a few changes: The turtles were maintained constantly in artificial sea water made up daily. The amino acids lysine, histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, and tryptophan were tested as before, and glycine was retested. Basic diet composition and preparation was the same as in the previous experiment, as was the experimental procedure.

Figure 4 compares the percentage of initial weight gained by the control to the percentage of initial weight gained by each of the deleted diet groups. This figure shows that only the glycine free group had a rate of growth approaching that of the control group. All other deleted groups had depressed rates of growth. As before, growth of the arginine-free group, while less than that of the control or glycine-free groups, was greater than amounts of growth obtained on the other deleted diets. All deleted groups except the glycine-free group showed increased rates of growth after being placed on the control diet. Only four of the initial 96 hatchlings died during the 4-week experimental period, indicating almost no disease.

FIGURE 4.
Rates Of Growth Of Hatchling Green Sea Turtles, Fed For
3 Weeks On Synthetic Diets Each Lacking A Different



Amino Acid (-----), Compared To Rate Of Growth
Hatchlings Fed The Control Diet (____). Number in () Indicates Number Of Hatchlings
Surviving Experimental Period.

Duncan's multiple-range test, when applied to the percentage weight gain values of each group at the end of the third week shows there to be no statistically significant difference between the control and glycine-free diet group. All other groups grew at a significantly slower rate than did the control or glycine-free groups. Arginine again occupied a more or less intermediate position, gaining less than the control or glycine-free group, but gaining more than the other deleted groups and being significantly different from all slower-growing groups except threonine.

These two experiments demonstrate that glutamic acid, aspartic acid, and glycine are nutritionally non-essential while lysine, histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, and tryptophan are nutritionally essential amino acids. The status of arginine is less clear. Its removal from the diet depressed growth in both experiments but not to the extent that deletions of other essential amino acids did. It appears that hatchling Green sea turtles may be able to synthesize arginine, but not in sufficient quantities to support maximum growth. Arginine should probably be considered as a semi-essential amino acid.

DISCUSSION OF RESULTS

It cannot be assumed that the qualitative dietary amino acid requirements of the adult GreenTurtle are the same as those of the hatchling. In nature the hatchling is presumed carnivorous during the first year and later becomes mainly herbivorous (Hirth, 1971). If herbivorous turtles utilize microbial action to digest cellulose, then their dietary requirements will depend upon the extent to which the intestinal flora modifies the dietary intake. Normally essential dietary components may be synthesized from non-essential elements by the intestinal micro-organisms. In such a case, the the animal's cells have not changed in their ability to synthesize amino acids, but the dietary requirement is met by microbial synthesis.

Such a system would also have a profound effect on quantitative amino acid requirements as well. Currently in large-scale turtle culture, rations fed are high in protein and relatively low in carbohydrate. On such diets the effect of microbial action in older turtles, if it does in fact exist, would probably be minimal.

One other factor should be discussed in a consideration of the relationship between hatchling and adult turtle amino acid requirements. The hatchling represents the most rapidly growing size class with rate of growth decreasing over time, until at maturity growth virtually ceases. With a decreasing rate of growth comes a corresponding decrease in demand for amino acids. The qualitative requirement in the absence of microbial action should remain the same, but the quantitative requirements could be radically different between the hatchling and the adult due to the difference in rate of growth. The amino acid arginine, if truly semi-essential, could become non-essential if the adult turtle could produce adequate quantities to meet its limited protein synthesis requirements.

Knowledge of the qualitative amino acid requirements of the Green sea turtle in itself has little practical value, since virtually every natural protein food source contains all 18 amino acids of nutritional significance, as well as many others. The next step in the study of the protein requirement of the Green sea turtle should be to determine the quantitative requirement for each of the essential amino acids. Research is also needed on required levels of protein, efficiency of

utilization of various carbohydrates, studies on lipid requirements, and vitamin requirements. As this information becomes known, rations can be formulated on a more factual basis using the least expensive ingredients to fulfil the requirements. The objective would be to utilize the protein fed for protein synthesis and to obtain energy for metabolic functions from the cheaper fat and carbohydrate sources. The possibility of microbial action in the digestive tract of herbivorous turtles should be investigated and, if found to exist (as expected), then studied to determine ways of utilizing this mechanism to lower feed costs. In much of the foregoing I have considered only manipulations of what goes in a turtle's mouth. This ignores the large factor of digestive tract efficiency and urinary excretion. Many standard nutritional techniques which would provide much useful information on turtle nutrition must await the development of a method for collecting total excreta during feeding trials.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the assistance of Dr. Robert Bustard, Australian National University, Mr. G. S. de Silva, Forest Department, Sabah, Malaysia; Dr. J. Schulz, Forest Service, Surinam; and Mariculture, Ltd., Grand Cayman Island, British West Indies in obtaining hatchling turtles. This work was supported in part by an NSF institutional grant to the University of Arizona and by funds supplied by Mariculture, Ltd. Thanks are given to Dr. Henry Schafer, Director of Escuela de Ciencias Maritimas y Tecnologia de Alimentos Gusymas, for generously providing laboratory space during the summer, 1973. This paper is a contribution of the Marine Biology Program of the University of Arizona.

LITERATURE CITED

- Borgstrom, C. 1962. Fish as Food. Vol. II. Nutrition, Sanitation, and Utilization. Academic Press, New York. 777 p.
- Borman, A., Wood, T. R. Black, H. C., Anderson, E. C., Oesterling, M. J., Womack, M. and Rose, W. C. 1946. The role of arginine in growth with some observations on the effects of argininic acid. Journal Biological Chemistry 171:585-594.
- CaIdwell, D. 1962. Growth measurements of young captive Atlantic sea turtles in temperate waters. Los Angeles County Museum Contributions in Science Number 50:1-8
- Coulson, R. A. and Hernandez I. 1964. Biochemistry of the Alligator - A Study of Metabolism in Slow Motion. Louisiana State University Press, Baton Rouge. 138 p.
- Coulson, R. A. and Hernandez, T. 1965. Amino acid metabolism in the alligator. Federation Proceedings. Federation American Societies for Experimental Biology 24:927-940.
- DeLong, D., Halver, J. and Mertz, E. 1959. Nutrition of summoned fishes. VII. Nitrogen supplements for chinook salmon diets. Journal of Nutrition 68:663-669.
- Harrison, T. 1955. The edible turtle (*Chelonia mydas*) in Borneo. 3. Young turtles (in captivity). Sarawak Museum Journal 6 (6): 633-640.
- Hirth, H. F. 1971. Synopsis of biological data on the green turtle *Chelonia mydas* (Linnaeus) 1758. FAO Fisheries Symposium (85):3.41.
- Pearse, A. S., Lepkovsky, S. and Hintze, L. 1925. The growth and chemical composition of three species of turtles fed on rations of pure foods. Journal Morphology and Physiology 41(1):191-216.
- United State Department of Agriculture. 1963. Composition of Foods. Agriculture Handbook Number 8. p. 56.
- Womack, M. and Rose, W. C. 1947. The role of proline, hydroxyproline, and glutamic acid in growth. Journal Biological Chemistry 171:37-50.