THE AMINO ACID REQUIREMENTS OF THE HATCHLING GREEN SEA TURTLE, *CHELONIA MYDAS*

by

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I hereby recommend that this dissertation prepared under my direction by James Raymon Wood Jr.

entitled The amino acid requirements of the hatchling green sea turtle, Chelonia mydas.

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

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After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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*This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.

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SIGNED: James R. Wallis

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ABSTRACT

The objective of this study was the determination of the essential amino acids required by the hatchling Green sea turtle, Chelonia mydas. The amino acid requirements of reptiles were totally unknown so this problem is significant as basic science. In addition, the study has applied value because the Green sea turtle is now being farmed on a trial basis.

The purified diet and the radioactively labeled carbon methods for the determination of essential amino acids are described and the advantages and disadvantages of each are discussed.

Qualitative amino acid requirements were determined by the purified diet method. The amino acid composition and the amounts of fat and carbohydrate in the initial purified diet were based upon the chemical composition of shrimp flesh. This diet contained 18.5% amino acid mixture, 1.5% dextrose, 2.5% vitamin mix, 1.4% salt mixture, 2.0% corn oil, 3.0% agar as a binder, and 71.1% distilled water.

This original diet was modified by (1) increasing the level of dextrose from 1.5% to 20.0%, (2) substituting potato starch for dextrose, (3) carboxymethyl cellulose replaced agar as the binder, and (4) the amino acid mixture was patterned after the composition of casein rather than shrimp.

Lysine, histidine, methionine, valine, leucine, isoleucine, threonine, tryptophan, and phenylalanine were found to be essential

amino acid for the hatchling sea turtle. Arginine is essential but a portion of this requirement is synthesized by the animal. Aspartic acid, glutamic acid, glycine, alanine, cystine, proline, serine, and tyrosine are non-essential.

The qualitative amino acid requirements of the hatchling sea turtle are identical to the requirements of the young rat and weanling pig. The hatchling amino acid requirement differs from that of the chick or teleost fish in that the hatchling can synthesize a portion of its arginine requirement. The role of the urea cycle in the synthesis of arginine is discussed and the presence or absence of this cycle is considered for mammals, birds, hatchling sea turtle, and teleost fish.

Glycine is an effective supplementary nitrogen source even when fed at high levels (11.4% of diet). The sea turtle is more tolerant to glycine than the chick since this level of glycine is highly toxic to the chick. Folic acid, niacin, and Vitamin B_{12} prevent glycine toxicity in the chick but the high tolerance to glycine of hatchling turtles cannot be accounted for by levels of folic acid in the turtle diet.

The practical value of results in the development of economical commercial rations is discussed.

CHAPTER 1

INTRODUCTION

Nutritionally essential amino acids are defined by Rose (1957) as "ones which cannot be synthesized by the species in question from materials ordinarily available to the cells at a rate commensurate with the needs for optimum growth." The term "semi-essential" amino acid has generally been used to indicate an amino acid which can be synthesized by the animal but not at a rate which allows optimum growth.

Determination of Essential Amino Acid Requirement

Since the role of protein intake is of such physiologic and economic importance in animal nutrition, there has been great interest in the determination of which amino acids are essential for various animal species, most of these being species of major economic importance. There are two methods for the determination of the nutritionally essential amino acid requirement. These are the purified diet method and the radioactive carbon labeling method.

Purified Diet Method

The purified diet method involves the development of a diet with a completely defined chemical composition, the make-up of which is such that the amount of the test substance can be controlled by the investigator. In the case of a purified diet designed for the determination of

essential amino acids, the amino acid composition of the diet must be known to the extent at least of the amount of the test amino acid.

The rationale of the purified diet method is that if growth response of animals fed a diet lacking a single dietary component is equal to the growth response of similar animals fed the same diet but including the missing dietary element, then the deleted substance is not nutritionally essential. If, however, the rate of growth of animals fed the deleted diet is not equal to that of animals fed a non-deleted diet, then the deleted component is nutritionally essential.

Since growth is often the criterion used to determine if a dietary component is nutritionally essential, it is important that the purified diet have a chemical composition which is palatable to the test animal and which, when consumed in adequate quantity, will promote growth and maintain the physical condition of the animals.

Early investigators of the role of individual amino acids in animal nutrition utilized crude purified diets containing protein sources deficient in only one or two amino acids, such as zein and gliadin, which could then be supplemented with the deficient amino acids to test their nutritional essentiality (Osborne and Mendel, 1914). Purified amino acid test diets were continually improved in the following years by the development of methods which allowed synthesis of pure individual amino acids. In 1935 the first successful amino acid test diet which utilized purified amino acids as the sole source of nitrogen was developed (McCoy, Meyer, and Rose, 1935-1936).

Radioactive Carbon Labeling Technique

The radioactive labeling technique involves the use of a radioactive carbon source such as C^{14} acetate or C^{14}-labeled glucose. The radioactive carbon source is either ingested or injected into the test animal. After a relatively short period of time (2-6 days) the animal is killed and the amino acids of the carcass are extracted. The individual amino acids are separated and the amount of radioactive carbon incorporated into the carbon skeleton of each amino acid is determined. Those amino acids which contain radioactive carbon in relatively high levels are considered non-essential while those containing little or no radioactive carbon are presumed essential. The rationale for this method is that synthesis of amino acids by the animal results in the incorporation of labeled carbon while those amino acids which the animal is incapable of synthesizing will be provided by the diet and therefore not be labeled. This method was initiated by Steele (1952) who demonstrated that the essential amino acids for the mouse (by the purified diet method) were labeled only slightly or not at all following the feeding of labeled carbohydrate. The non-essential amino acids were heavily labeled. The exceptions were the non-essential amino acid tyrosine, which was unlabeled indicating that this amino acid is synthesized solely from the essential amino acid phenylalanine; and methionine, an essential amino acid which had a level of carbon labeling higher than the other essential amino acids due to the labeling of its active methyl group. This method has been used by several investigators in the determination of essential amino acids required by the

blowfly (Kasting and McGinnis, 1958), flatfish (Cowey, Adron, and Blair, 1970), and the prawn (Cowey and Forster, 1971).

Advantages and Disadvantages of Methods

Both the purified diet method and the radioactive carbon labeling method have advantages as well as disadvantages. The main disadvantage of the purified diet method is that a suitable test diet must be developed for each test species. This often involves an intensive period of preliminary experimentation before the correct diet is formulated. The purified diet method also has the disadvantages of requiring relatively large numbers of experimental animals and necessitating an experimental period of relatively long duration, the actual length of experimental period depending upon the test criterion, animal species being used, and the substance being tested. The major advantage of the purified diet method is that once the diet has been developed then this same diet can be utilized not only for the determination of which dietary elements are essential (the qualitative requirement) but the actual amounts required of each dietary component (the quantitative requirement). The major advantages of using the radioactive carbon labeling method are the small number of animals required and the shorter experimental period. The disadvantages are the requirement for sophisticated chemical and analytical equipment and the fact that this method gives information only on the qualitative requirement and provides little information on the quantitative requirement.

Statement of Research Problem

The problem chosen for study in this research program has been the determination of the nutritionally essential amino acids required by the hatchling Green sea turtle, Chelonia mydas. This research was initiated for two reasons: (1) it would be the first time that the nutritionally essential amino acids required by a reptile had been determined, and (2) on an applied level, information on the basic amino acid requirement of this species in its infancy as a "domestic" and captive cultured animal would be very important in laying the groundwork for future protein research leading to the development of economical commercial rations. The purified diet method was chosen for this research since, once the purified diet was developed, it could be used to determine both the qualitative and quantitative amino acid requirements.

Previous Research

Previous research on the nutritional requirements of reptiles is virtually non-existent. Pearse, Lepkovsky, and Hintze (1925) conducted a dietary study on a painted turtle, a gopher tortoise, and a terrapin. The turtles 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 homeothermic animals, which turns out to be the case.

Coulson and Hernandez have conducted studies on amino acid metabolism in the alligator, caiman, and turtle. The most common

method employed by these authors has been the monitoring of plasma and tissue amino acid levels under various conditions. They have shown that glycine, alanine, glutamine, and leucine are natural precursors of urinary NH_3 in the alligator (Coulson and Hernandez, 1959). Coulson and Hernandez (1964) 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). The amino acids essential for the rat are assumed essential for the caiman by Herbert, Coulson, and Hernandez (1966). Glutamine, alanine, and glycine were shown to be responsible for 80% of the rise in plasma free-amino acids after feeding, with these 3 amino acids being the major nitrogen carriers in the caiman (Coulson and Hernandez, 1967).

CHAPTER 2

MATERIALS AND METHODS

The determination of the qualitative amino acid requirements of the Green sea turtle actually consisted of two parts. First it was necessary to develop a synthetic test diet which would promote rapid growth. Then this diet was used to determine the essential amino acids. The first 12 experiments involved either trial-and-error experimentation on diet composition or were experiments designed to determine amino acid requirements which failed due to diet composition or disease, these 12 experiments are only summarized here. Complete details of methods, materials, and results are given in Appendix 1. Materials and methods for experiments 13-15, which yielded the actual information for determination of the qualitative amino acid requirements, are given in detail. Unless otherwise noted, all turtles were hatchling Chelonia mydas, the Green sea turtle.

Experiments 1-12

Experiment 1

The amino acid composition and the amounts of fat and carbohydrate in the initial purified diet were based upon the chemical composition of shrimp flesh. It contained 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. I attempted to

determine the essentiality of 6 amino acids. All groups fed the synthetic diets failed to gain weight. It was possible that high acidity (resulting from the free amino acid mixture) effected palatability. Therefore I decided to test the effect of different levels of pH and amino acid mixtures.

Experiment 2

Hatchlings were fed diets having pH values of 3, 5, or 7 and containing approximately 8, 16, or 29 percent amino acid mixture. The increased protein level enhanced palatability, with this effect being directly related to diet pH. Since one group (pH 7, 26% amino acid mix) ingested large quantities of diet but lost weight, I concluded that the diet was still not suitable. The possibility of inadequate energy in the diet was next considered.

Experiment 3

Groups of hatchlings were fed diets containing either casein or amino acid mixture as the protein source, with the level of dextrose increased from 1.5% to 20.0% of the diet. Increasing the amount of dextrose in the amino-acid-containing diet slightly improved the growth-promoting abilities of the diet, but rapid growth was not achieved. Groups fed the casein-containing diet had growth rates exceeding those of groups fed the amino-acid-containing diet.

Experiment 4

Some hatchlings were fed an amino acid diet with sodium bicarbonate added (to reduce diet acidity) and with 30% dextrose. Other

groups received diets containing either 7 or 14% casein and 24% dextrose. Raising the level of dextrose had no apparent effect on growth. The effect of sodium bicarbonate was not clear. The lower level casein diet resulted in decreased growth when compared to the high level diet.

Experiment 5

In the absence of adequate supply of Green turtle hatchlings, Pacific Ridley hatchlings (Lepicochelys olivacea) were used in further series of tests to determine the effects of: (1) a mixture of casein and amino acids as the protein source; (2) substituting potato starch for dextrose; (3) increasing the proportion of corn oil, while decreasing carbohydrate; (4) addition of sodium bicarbonate as a neutralizer, and (5) feeding 10% or 20% dextrose. Growth in all groups fed the synthetic diets was similar, with the group fed the diet containing potato starch having the best growth. I decided to use potato starch in future diets. The effect of the other variables tested could not be determined.

Experiment 6

Diets containing either 18% or 36% amino acid mixture with 20% potato starch were fed to Pacific Ridley hatchlings. The group fed the diet containing 36% amino acid mixture ate approximately twice as much as the group fed the 18% amino acid diet. However, both groups lost weight. This growth failure could not be explained. Salt mix used in the diet was modified by addition of sodium, potassium, phosphorous, and chlorine to bring the mineral composition of the diet closer to that of shrimp flesh.

Experiment 7

Hatchlings were fed diets containing 18% amino acid mixture, 20% potato starch, the modified salt mixture, and the other dietary ingredients, in further tests of the essentiality of 6 amino acids. Results were inconclusive, but suggested that proline is non-essential; threonine and isoleucine appeared to be essential.

Experiment 8

Loggerhead hatchlings (Caretta caretta gigas) were fed diets containing various amino acid mixtures as the protein source: (1) the mixture reported from chick studies, (2) the mixture reported from salmon studies, (3) a modification of the mixture used in experiments 1 - 7, or (4) the mixture used in Experiments 1 - 7, unmodified. The group receiving the chick diet amino acid mixture lost weight. Weight gain on the other three amino-acid-containing diets was poor, with no apparent difference in the effect of the chosen mixtures. I decided to continue using the amino acid mixture used in previous experiments. Examination of the intestinal tract of one turtle from each of the synthetic diet fed groups indicated that the agar used to bind the diet was accumulating and blocking the gut.

Experiment 9

Groups of hatchlings were fed diets containing 18% amino acid mixture, 20% potato starch, and the other dietary ingredients. These diets were bound using either agar, carboxymethyl cellulose (CMC), or gelatin. Two other groups were fed diets containing either hydrolyzed or unhydrolyzed casein and were bound with CMC. The CMC-bound amino

acid test was approximately twice as effective as diets bound with gelatin or agar. Unhydrolyzed casein appeared to be a better protein source than hydrolyzed casein, however, growth on the hydrolyzed casein diet was greater than that seen on the amino acid mixture (patterned after shrimp flesh). I decided to utilize CMC and an amino acid mixture patterned after casein rather than shrimp in future diets.

Experiment 10

Using diets bound with CMC and containing an amino acid mixture patterned after casein, the essentiality of 12 amino acids was tested. Disease caused 44% mortality, so no conclusions could be reached.

Experiment 11

Using diets identical to those in Experiment 10, the essentiality of 10 amino acids was tested. At the end of the third week, only one group was gaining weight, indicating that the experiment was going to fail. The amount of amino acid mixture in the diets was increased from 18-36% and the potato starch level was decreased from 20-10%. This resulted in impressive rates of growth in those groups fed diets lacking amino acids which were traditionally non-essential. Since it was necessary to terminate the experiment after only one week at the raised amino acid levels, no conclusions were drawn.

Experiment 12

This experiment tested the essentiality of 18 amino acids, using diets containing 36% amino acid mixture and 10% potato starch.

Severe disease resulted in a 68% mortality. However, groups fed diets lacking either serine, glycine, tyrosine, cystine, alanine, or proline all had relatively good rates of growth and mortality was lower than in the other groups. This suggested that the above 6 amino acids were non-essential.

Experiments 13-15

Experiment 13

The main objective of this experiment was to retest the 6 amino acids which had seemed to be nutritionally non-essential in Experiment 12. The effectiveness of glycine when used as a nitrogen supplement was also to be determined.

The turtles used were survivors of the last experiment. The remaining turtles from Experiment 12 were brought back to Tucson from Guaymas and maintained on chopped shrimp for 10 days. At the end of this period the turtles were weighed and the 16 turtles showing the greatest weight gain during the 10 day period were divided into 4 groups of 4 turtles each. The turtles were already tagged. Individuals of each group were weighed at the beginning of each week.

The water system used in this experiment consisted of two sets of 12 liter plastic dish pans (round). One set contained artificial sea water (Rila brand) and the other set contained fresh (tap) water. The salt water in each tub was filtered using a Biozonic 3-stage filter modified to fit the shallower tubs. The turtles were transferred in the morning from the salt water to the tubs containing fresh water where the turtles were fed during the day. In the evening the turtles

were placed back in the salt water tubs and the fresh water tubs were emptied and rinsed thoroughly and then refilled for the next day's feeding. The filters were cleaned once each week and the salt water was replaced at the end of the second week. The water temperature varied between 25-29°C with the salt water salinity being approximately 32°/oo.

Group 1 was fed Diet 104 containing 36% of an L-alanine-free amino acid mixture plus the other dietary ingredients. The composition of this diet is given by Table 1, being modified only in that the deleted L-alanine was replaced with 1.32 grams of diammonium citrate. Group 2 received Diet 105 which contained 18% of an amino acid mixture lacking L-alanine, L-proline, glycine, L-cystine, L-tyrosine, and L-serine. Group 3 received Diet 106 which was similar to Diet 105 except that the level of amino acid mixture used was increased to 36%. Group 4 was fed Diet 107. This diet was similar to Diet 105 except that it was supplemented with glycine. The amount of glycine added provided the nitrogen equivalent to an additional 18% amino acid mixture. The composition of Diets 105-107 is given by Table 2. Diet preparation and feeding was as in the last experiment.

Experiment 14

The purpose of this experiment was to determine if the 12 test amino acids not tested in Experiment 13 were nutritionally essential for the hatchling Green sea turtle.

The turtles used in this experiment were hatchling Green sea turtles provided by Mariculture, Ltd., Grand Cayman Island. They had hatched from eggs laid on the artificial nesting beach at Mariculture,

Table 1. Chemical composition of Diet 085. Number in () indicates amount of diammonium citrate used to replace each deleted amino acid.

Ingredient	gm/100 gm of diet	
L-Lysine • HCl	1.44	(1.78)
L-Histidine • HCl • H_2O	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	(3.64)
Glycine	1.28	(1.94)
L-Crystine	.28	(0.26)
L-Valine	2.02	(1.96)
L-Methionine	.70	(0.52)
L-Alanine	1.04	(1.32)
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	.36	(0.36)
Total	36.00	
Potato Starch	10.00	
Vitamin Diet Fortification Mixture	2.50	
Hawk Oser Salt Mixture No. 3	1.40	
Potassium Phosphate Monobasic	.40	
Sodium Chloride	.20	
Corn Oil	2.00	
Carboxymethyl Cellulose Sodium	5.00	
Distilled Water	42.50	

Table 2. Chemical composition of Diets 105-107

Ingredient	gm/100 gm of diet		
	Diet 105	Diet 106	Diet 107
L-Lysine • HCl	1.01	2.02	1.01
L-Histidine • HCl . H_2O	.72	1.44	.72
L-Arginine • HCl	.94	1.88	.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	.49	.98	.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	.25	.50	.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	1.40	1.40
Potassium Phosphate Monobasic	.40	.40	.40
Sodium Chloride	.20	.20	.20
Corn Oil	2.00	2.00	2.00
Carboxymethyl Cellulose Sodium	5.00	5.00	5.00
Distilled Water	42.52	42.54	42.52

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

Ltd. Since the breeding population contains turtles from Ascension Island, Costa Rica, and Surinam it was not possible to determine the actual genetic population from which the hatchlings came. The hatchlings were placed in artificial sea water during the 4 days between time of arrival in Tucson and the beginning of the experiment. The turtles were divided into 13 groups of 8 hatchlings each and then tagged and individually weighed. The turtles appeared in good condition with only a few hatchlings showing areas of the common skin infection. The yolk scar had not completely closed on most of the hatchlings. The turtles were weighed at the beginning of each week of the experiment.

The water system was the same as in the last experiment with the turtles being fed during the day in tubs of fresh water and then being transferred in the evening to tubs containing artificial sea water. Water temperature varied between 24-30°C during the experiment with the low occurring in the morning and the high being an evening temperature. Salinity varied between 32-36°/oo.

All 13 groups received synthetic diets. Group 1 was fed the control diet (Diet 108) containing all the amino acids used in previous control diets except L-alanine, which had been deleted since groups fed diets lacking this amino acid in previous experiments had done consistently well. Groups 2-13 received modifications of Diet 108, in each case the modification consisting of the removal of a single amino acid and the replacement of the deleted amino acid weight for weight with "Alphacel," a ground cellulose powder. It was decided to discontinue the use of diammonium citrate after learning that this compound had been shown to have a negative effect on the growth of

chinook salmon (DeLong, Halver, and Mertz, 1959). The ground cellulose was used so that concentrations of dietary ingredients would be equal in each of the test diets. Groups 2-13 received the following diets: Group 2, L-lysine-free Diet 109; Group 3, L-histidine-free Diet 110; Group 4, L-arginine-free Diet 111; Group 5, L-aspartic acid-free Diet 112; Group 6, L-threonine-free Diet 113; Group 7, L-glutamic acid-free Diet 114; Group 8, L-valine-free Diet 115; Group 9, L-methionine-free Diet 116; Group 10, L-isoleucine-free Diet 117; Group 11, L-leucine-free Diet 118; Group 12, L-phenylalanine-free Diet 119; and Group 13, L-tryptophan-free Diet 120. All diets lacked L-alanine. The chemical composition of Diet 108 is given in Table 3. Diet preparation and feeding was the same as in Experiment 13. Turtles were fed for 6 days each week, not being fed the day prior to weighing. During the last week of the experiment, Groups 2-13 each received the control diet (Diet 108) in order to observe the response of each group to having the deleted amino acid returned to the diet.

Experiment 15

The purpose of this experiment was to retest those 10 amino acids found to be nutritionally essential or semi-essential in the preceding experiment. The amino acid glycine, found to be non-essential in previous experiments was retested to confirm its non-essential status and to provide a group in addition to the control for contrast with groups receiving diets lacking an essential amino acid.

Table 3. Chemical composition of Diet 108

Ingredient		gm/100 gm of diet*
L-Lysine • HCl		1.44
L-Histidine • HCl • H_2O		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-Crystine		.28
L-Valine		2.02
L-Methionine		.70
L-Isoleucine		1.52
L-Leucine		3.26
L-Tyrosine		1.96
L-Phenylalanine		1.70
L-Tryptophan		.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		.40
Sodium Chloride		.20
Corn Oil		2.00
Carboxymethyl Cellulose Sodium		5.00
Distilled Water		43.54

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

Turtles for this experiment were again provided by Mariculture, Ltd., Grand Cayman Island. Hatchlings were obtained from eggs collected by Mariculture, Ltd. at Tortuguerro Beach, Costa Rica. The turtles were kept in artificial sea water for the 2 day period between arrival and the beginning of the experiment. The turtles appeared to be in excellent condition with the umbilical scars fairly well closed. The turtles were divided into 12 groups of 8 hatchlings each and then individually tagged and weighed. The turtles were weighed at the beginning of each week of the experiment.

The water system used in this experiment differed from that used in Experiment 14 in that the hatchlings were maintained constantly in artificial sea water. In the previous two experiments the test animals had been transferred each day from salt water to fresh water and back to salt water again. While there were no indications that this was placing the animals in daily osmotic shock, I felt that in this critical experiment only salt water should be used to be assured of accurate results. The hatchlings of each group were kept in a round plastic dish pan identical to those used in the previous two experiments. Each pan contained approximately 7 cm of artificial sea water. In the mornings each group received the first of its daily feedings, receiving additional diet several times during the day as needed. In the evenings the turtles of each group were removed and rinsed with tap water. The uneaten food, if any, was removed and weighed. The dish pans were rinsed with hot tap water and then refilled with fresh artificial sea water and the turtles placed back in the tub. The water

temperature ranged from 23° to 29°C during the experimental period, with the morning water temperature usually being about 4°C lower than the evening temperature. Since fresh salt water was made up each day, the salinity varied from day to day with an average of approximately 30°/oo but with a range between 26-37°/oo. The water remained very clear through most of each day, but by evening visibility was greatly reduced. This system seemed to give very satisfactory results with relatively little work since there were no filters to clean.

The diets used in this experiment were the same as those used in Experiment 14 with the exception that in this experiment a glycine-free diet was fed in place of either L-glutamic acid-free or L-aspartic acid-free diets. The control diet (Diet 121) was identical to Diet 108, with the deleted diets being single amino acid deletions of this basic diet. The groups received the following diets: Group 1, control Diet 121; Group 2, L-lysine-free Diet 122; Group 3, L-histidine-free Diet 123; Group 4, L-arginine-free Diet 124; Group 5, L-threonine-free Diet 125; Group 6, L-valine-free Diet 126; Group 7, L-methionine-free Diet 127; Group 8, L-isoleucine-free Diet 128; Group 9, L-leucine-free Diet 129; Group 10, L-phenylalanine-free Diet 130; Group 11, L-tryptophan-free Diet 131; and Group 12, glycine-free Diet 132. The composition of Diet 121 (Diet 108) is given by Table 3. Note that all diets are L-alanine-free with ground cellulose replacing each deleted amino acid. During the fourth week of the experiment, all groups received the control diet. Diet preparation and feeding procedure was the same as for Experiment 14 with the turtles receiving food only 6 days a week, not being fed the day prior to weighing.

CHAPTER 3

RESULTS

Experiment 13

Table 4 gives the weights of the turtles during the 4 weeks of the experiment. As can be seen from this table, only 3 turtles died. The average weight change as percent of initial weight is given for each group by Figure 1. Group 1 demonstrated the most rapid weight gain. Group 2, fed the low level of the deleted amino acid mixture (Diet 105) did not do well, losing weight by the end of the experiment. Group 3, fed the 36% level of the deleted amino acid mixture, gained about 22% of its initial weight while Group 4, fed the low amino acid level supplemented with glycine did almost as well as Group 3.

I felt that several important facts were proven by this experiment. The good rate of growth achieved on Diet 104 by Group 1 showed L-alanine to be nutritionally non-essential. While the rate of growth obtained on Diet 106 by Group 3 was less than that of Group 1, I concluded that the deleted amino acids L-serine, L-proline, L-cystine, glycine, L-alanine, and L-tyrosine were nutritionally non-essential, since turtles in this group gained an average of 16 grams each. These animals could not have gained this amount of weight during the 4 week period if any of the missing amino acids were nutritionally essential. The failure of Group 3 to grow at a rate equal to Group 1 can possibly

Table 4. Individual weights in grams of turtles in each group of Experiment 13 during experimental period

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 1					
#497	74.9	79.4	91.1	98.8	104.1
#527	99.1	104.1	116.6	128.4	133.2
#532	56.3	60.1	71.7	82.2	91.4
#556	50.7	39.2	45.2	dead	dead
Group 2					
#488	91.3	98.0	104.0	dead	dead
#531	77.6	74.7	86.4	87.9	83.1
#551	54.0	43.0	dead	dead	dead
#592	63.8	61.8	62.4	58.3	56.3
Group 3					
#485	73.9	77.8	84.3	87.2	91.0
#550	62.4	62.0	65.6	66.2	66.7
#620	67.3	71.3	78.3	84.9	88.4
#626	81.9	85.6	95.5	102.0	105.9
Group 4					
#521	70.0	68.8	73.3	77.9	83.4
#549	53.6	57.2	58.5	63.2	65.6
#578	86.3	89.6	96.4	97.3	102.9
#612	113.0	116.1	130.3	136.5	141.7

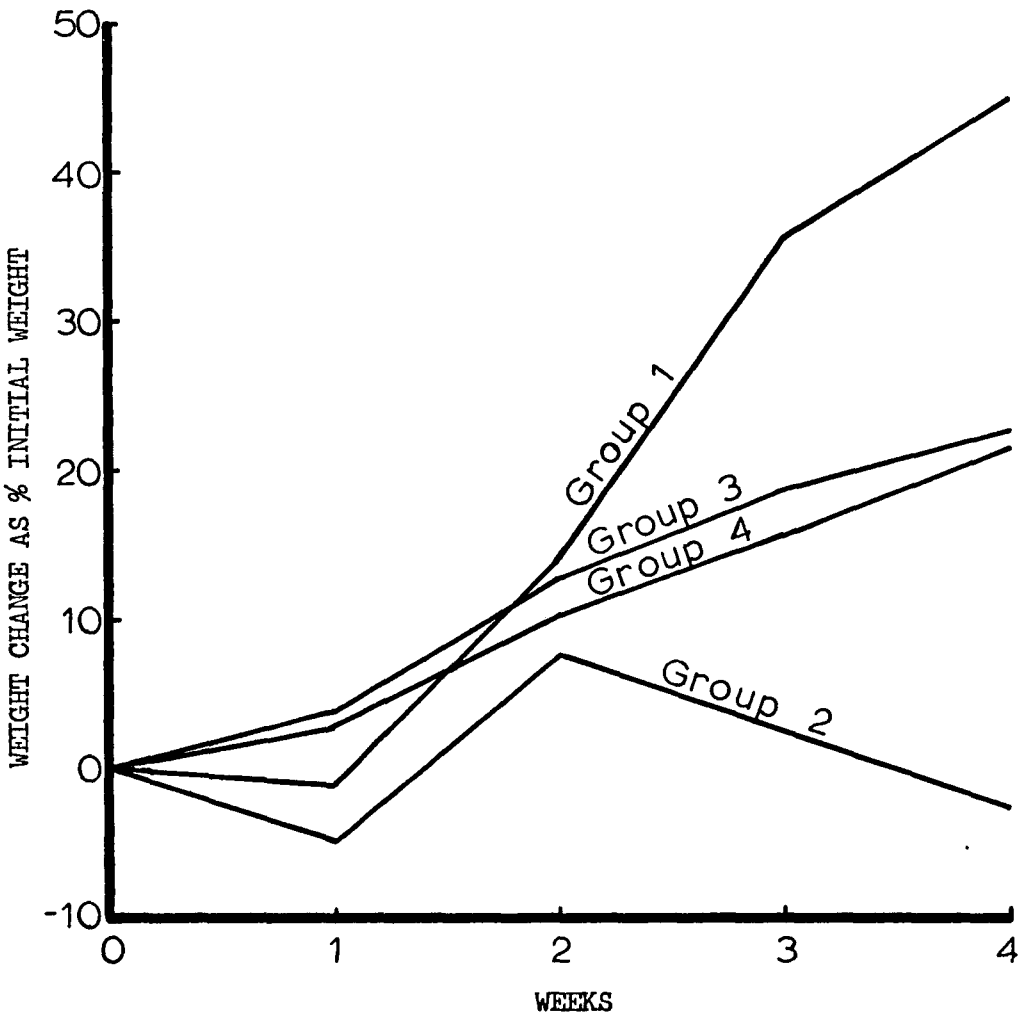


Figure 1. Average weight change of turtles in each group of Experiment 13 as a percentage of their initial weight

be explained in the same way that Rose, Oesterling, and Womack (1948) explained the decreased rate of growth of rats fed diets missing 9 non-essential amino acids. They suggested that while the amino acids were non-essential, they had to be synthesized if not provided by the diet and that the rat perhaps could not synthesize all nine of the missing amino acids at a rate commensurate with maximum growth. Glycine is an efficient nitrogen supplement with the ability to replace one-half the amino acid nitrogen contained in Diet 106 without effecting rate of growth. The failure of Group 2 to gain weight indicates that the 18% level of the deleted amino acid mixture is incapable of promoting growth due either to amino acid level or pattern of amino acid composition.

In summary, results indicated that proline, alanine, cystine, serine, tyrosine, and glycine are non-essential amino acids for the hatchling Green sea turtle. Glycine is a relatively efficient nitrogen supplement.

Experiment 14

The weights of the hatchlings during the experiment are given in Table 5. This table indicates that towards the last of the experiment a relatively large number of hatchlings had died. Total mortality was 32%. Figure 2 graphically compares the weight change as percent of initial weight values for Groups 2-13 with the rate of growth for the control, Group 1. As can be seen from this figure, each group gained weight during the first week of the experiment. This gain was expected even for those groups receiving diets lacking essential amino acids, since all the hatchlings initially carried into the experiment with

Table 5. Individual weights in grams of turtles in each group of Experiment 14 during experimental period

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 1					
#642	25.9	30.3	34.6	34.3	dead
#643	24.3	31.5	37.0	38.4	42.7
#644	23.4	27.8	29.6	32.4	34.1
#645	24.7	30.8	34.2	30.9	34.0
#646	25.4	28.7	34.5	36.9	41.2
#647	23.3	28.8	32.4	33.9	dead
#648	26.5	30.4	35.7	36.5	42.4
#649	25.7	32.7	37.0	40.1	43.4
Group 2					
#650	25.9	28.8	dead	dead	dead
#651	24.5	26.8	29.6	27.1	28.2
#652	29.2	31.8	32.2	33.9	dead
#653	22.4	24.4	24.6	23.8	23.7
#654	22.7	24.5	23.8	25.6	28.3
#655	21.7	23.9	22.8	22.5	dead
#656	23.7	25.4	25.0	21.6	dead
#657	24.8	26.6	27.1	28.6	25.4
Group 3					
#658	28.2	32.8	34.2	34.6	38.0
#659	28.5	33.8	33.8	35.2	dead
#660	26.6	28.3	27.5	dead	dead
#661	28.3	31.6	33.9	31.6	31.4
#662	24.2	27.4	29.4	27.9	32.3
#663	23.5	28.0	28.3	27.4	29.8
#664	24.6	27.6	29.3	28.7	32.4
#665	24.2	27.7	28.6	26.1	dead
Group 4					
#666	24.5	30.8	33.4	35.6	36.8
#667	24.3	29.7	33.0	35.5	36.6
#668	26.9	31.2	32.1	33.6	39.1
#669	24.1	27.4	29.8	31.3	36.6
#670	24.0	30.2	33.5	31.8	35.9
#671	28.9	30.7	32.9	34.0	35.1
#672	23.4	27.1	28.7	30.5	31.0
#673	25.7	30.2	32.7	dead	dead

Table 5, Weights of turtles in Experiment 14, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 5					
#674	25.3	31.1	33.8	31.4	32.5
#675	25.0	30.9	32.5	dead	dead
#676	28.6	32.8	33.2	dead	dead
#677	28.0	32.4	39.9	45.9	47.3
#678	30.2	38.0	41.5	40.0	33.1
#679	23.0	28.3	29.2	dead	dead
#680	23.9	26.0	30.4	33.2	33.2
#681	25.1	33.0	39.0	42.1	43.0
Group 6					
#682	27.5	29.2	28.5	28.8	28.9
#683	22.7	24.1	24.5	24.8	28.0
#684	23.3	25.9	27.6	26.3	28.5
#685	23.5	25.9	26.2	24.5	dead
#686	23.2	25.3	26.4	27.2	26.9
#687	21.9	24.0	24.7	21.0	dead
#688	27.3	28.4	25.1	23.9	dead
#689	25.8	28.2	26.5	23.3	dead
Group 7					
#690	25.8	31.2	34.2	37.8	41.6
#691	24.9	30.1	35.0	37.2	31.4
#692	26.9	33.7	33.6	34.8	37.8
#693	25.1	28.5	31.9	30.2	dead
#694	28.0	36.5	43.1	41.2	46.5
#695	23.8	27.6	32.0	36.2	38.8
#696	22.5	27.2	32.6	35.8	34.0
#697	24.4	29.2	32.4	34.2	34.0
Group 8					
#698	25.6	23.2	dead	dead	dead
#699	29.0	30.2	dead	dead	dead
#700	25.1	27.3	28.3	29.4	28.4
#701	24.1	25.0	25.6	24.5	dead
#702	28.8	29.3	28.0	dead	dead
#703	26.5	29.5	24.0	dead	dead
#704	23.5	26.7	26.4	25.8	dead
#705	22.3	24.2	23.3	dead	dead

Table 5, Weights of turtles in Experiment 14, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 9					
#706	24.8	27.7	27.9	28.0	28.8
#707	23.8	25.3	27.1	25.0	27.9
#708	25.7	29.1	28.0	28.0	27.5
#709	24.5	26.9	29.5	26.7	31.0
#710	23.0	25.6	25.3	23.8	26.1
#711	25.8	28.3	29.8	29.9	32.2
#712	28.0	30.2	32.0	30.8	dead
#713	28.7	29.5	29.2	dead	dead
Group 10					
#714	28.8	31.4	31.6	28.2	dead
#715	23.2	26.5	24.3	22.8	24.5
#716	24.2	26.7	25.1	22.8	23.3
#717	26.4	28.6	28.5	28.4	31.0
#718	24.0	25.9	24.4	22.1	dead
#719	23.1	25.1	26.1	25.5	24.7
#720	28.2	31.3	27.4	22.8	dead
#721	25.0	27.4	28.1	27.7	30.9
Group 11					
#722	24.6	28.1	28.8	30.1	31.8
#723	23.8	26.7	28.1	25.4	22.0
#724	27.3	27.8	27.9	27.7	25.2
#725	25.0	26.7	28.4	28.4	31.3
#726	23.8	27.9	28.1	26.6	26.4
#727	24.4	28.0	28.2	27.1	23.3
#728	23.1	28.0	27.4	26.3	25.9
#729	22.6	25.3	27.0	26.7	27.3
Group 12					
#730	26.0	30.0	29.7	30.0	32.0
#731	21.5	23.6	23.9	23.9	27.0
#732	24.8	27.8	29.0	29.4	32.4
#733	23.0	26.1	26.2	25.8	29.7
#734	22.7	25.4	26.5	27.1	27.6
#735	25.1	27.4	29.3	31.5	33.7
#736	26.6	27.2	27.5	30.0	30.7
#737	22.9	24.5	25.8	24.9	25.8

Table 5, Weights of turtles in Experiment 14, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 13					
#738	28.3	30.9	31.1	30.7	dead
#739	23.5	25.9	27.5	29.1	32.1
#740	27.7	30.4	31.0	30.6	dead
#741	26.2	29.2	29.3	29.4	35.8
#742	27.5	30.5	31.2	32.5	38.4
#743	28.3	30.7	31.7	32.3	34.0
#744	23.8	27.0	28.6	28.0	30.0
#745	26.2	27.4	27.6	dead	dead

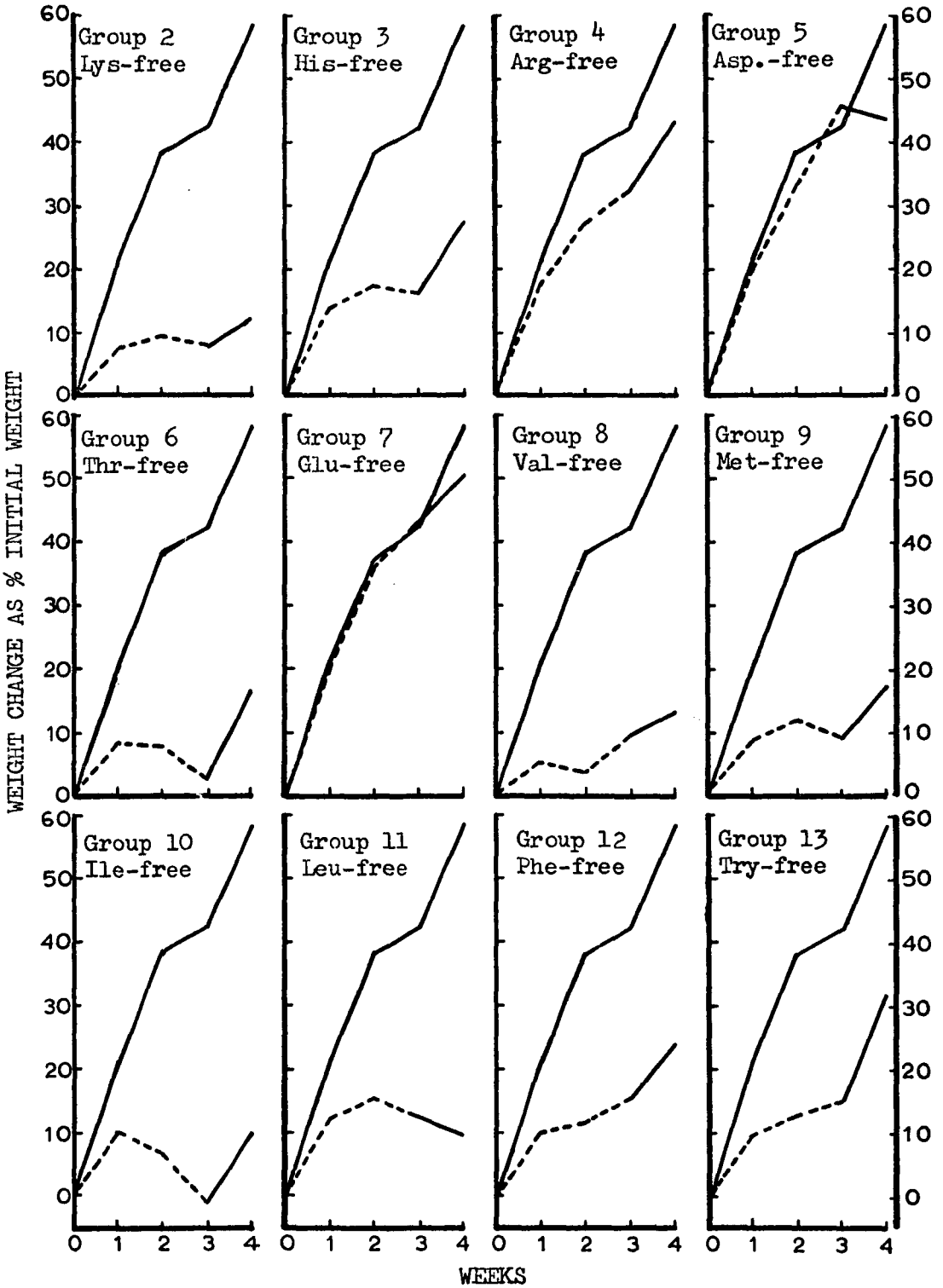


Figure 2. Average weight change of turtles in each group of Experiment 14 as a percentage of their initial weight. (———) Control Diet 108; (------) Deleted diet.

them stored yolk. During the second and third weeks, several groups had the rate of growth decline sharply, in most cases losing weight during the third week. This was taken to indicate that major yolk stores were depleted by the end of the second week.

Duncan's new multiple-range test (Steel and Torrie, 1960) was applied to the individual percent of initial weight gain values at the end of the third week. The mean percent of initial weight gain value for each group with an appropriate Duncan subscript letter(s) is given by Table 6. Groups having even a single subscript letter in common are not statistically significantly different at the .05 level. From this table it can be seen that there was no statistically significant difference between the control (Group 1) and Group 5 (L-aspartic acid-free diet group) and Group 7 (L-glutamic acid-free diet group). The control was significantly different from all other groups indicating that these other 10 groups were fed diets lacking amino acids of an essential nature. Group 4, the L-arginine-free diet group, occupied an intermediate position, being significantly different from all other groups with a growth response less than that shown by Groups 1, 5, and 7 but more than that shown by Groups 2, 3, 6, 8, 9, 10, 11, 12, and 13. It would seem that L-arginine is perhaps a nutritionally semi-essential amino acid, with the hatchling turtle being able to synthesize some of this amino acid but not enough for maximum growth.

During the last week of the experiment, all groups which had received diets lacking amino acids except Group 8 and Group 11 responded in a positive manner to the replacement of the essential amino acid in the diet. Why Groups 8 and 11 did not respond is not known unless it

Table 6. Average percent of initial weight change values for each group of Experiment 14 at the end of the third week with subscript letter(s) for each group showing results of Duncan's multiple-range test. Groups with even a single subscript letter in common are not statistically significantly different at the P=.05 level

Group	Mean %	Subscript
Group 5	45.40	A
Group 7	43.01	A
Group 1	42.31	A
Group 4	32.37	B
Group 3	16.33	C
Group 12	15.46	C
Group 13	14.98	C
Group 11	12.38	CD
Group 8	9.50	CD
Group 9	9.31	CD
Group 2	7.97	CD
Group 6	5.85	CD
Group 10	3.80	D

was due to disease. During this same period Groups 5 and 7 showed a decline in growth rate.

In summary, this experiment indicated that L-aspartic acid and L-glutamic acid are nutritionally non-essential; L-arginine is a nutritionally semi-essential; and the amino acids L-lysine, L-histidine, and L-threonine, L-valine, L-methionine, L-isoleucine, L-leucine, L-phenylalanine, and L-tryptophan are nutritionally essential for the hatchling Green sea turtle. Since disease became something of a problem during the last of this experiment, it was decided to retest those amino acids considered essential or semi-essential in the next experiment.

Experiment 15

The weights of the hatchlings during the experiment are given by Table 7. This table shows that only 4 hatchlings died during the experiment, indicating that disease was virtually no problem. Figure 3 compares the rates of growth, expressed as percent of initial weight gained, for each group receiving a deleted diet to the rate of growth of the control group (Group 1).

The individual percent of initial weight gain values for each group at the end of the third week were subjected to Duncan's new multiple-range test to determine if any statistically significant differences exist between the 12 groups. The mean values for each group are given with the appropriate subscript letter by Table 8. Groups having even a single subscript letter in common are not statistically significantly different at the .05 level. From this table it can be seen that no differences exist between the control (Group 1) and the

Table 7. Individual weights in grams of turtles in each group of Experiment 15 during experimental period

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 1					
#760	25.2	29.8	34.4	41.2	45.6
#761	22.5	26.3	28.5	30.2	28.9
#762	24.2	27.9	29.9	30.2	32.7
#763	26.4	30.8	34.7	38.0	41.0
#764	25.4	30.5	33.6	36.0	40.5
#765	24.6	27.2	30.1	30.9	33.9
#766	23.2	27.2	28.9	30.1	32 4
#767	26.6	30.7	35.4	38.7	44.7
Group 2					
#768	23.4	23.9	24.2	23.1	26.4
#769	26.5	30.6	29.3	28.3	32.6
#770	25.9	27.5	30.0	30.2	32.9
#771	24.7	26.2	27.5	30.8	31.2
#772	24.4	26.4	27.4	27.9	31.0
#773	24.6	26.8	27.2	28.7	32.5
#774	24.1	25.8	28.4	30.9	dead
#775	23.5	24.5	25.7	26.9	30.8
Group 3					
#776	28.6	32.2	34.4	33.2	34.2
#777	24.1	26.5	28.8	28.6	33.0
#778	28.7	31.5	32.5	33.2	36.3
#779	26.0	28.4	29.6	30.0	34.2
#780	25.1	26.9	27.8	28.1	32.0
#781	25.4	27.6	27.8	25.8	28.2
#782	24.2	27.9	29.5	31.5	34.2
#783	27.9	29.1	31.9	31.3	34.6
Group 4					
#784	26.1	28.5	30.9	31.2	35.1
#785	27.2	30.9	33.2	32.8	35.4
#786	25.0	29.4	31.0	30.4	33.6
#787	25.4	27.9	32.0	32.5	34.5
#788	27.1	31.7	35.2	36.8	38.6
#789	24.0	25.8	28.0	30.5	32.8
#790	28.0	30.7	32.9	33.7	36.3
#791	26.5	30.2	33.3	34.2	36.8

Table 7, Weights of turtles in Experiment 15, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 9					
#824	24.4	26.1	26.0	25.1	26.8
#825	23.4	25.2	26.0	25.5	28.3
#826	23.1	24.6	25.0	24.3	24.2
#827	30.2	34.3	37.9	36.0	39.0
#828	25.7	28.0	29.1	28.0	30.2
#829	24.9	26.6	27.9	27.8	27.8
#830	24.7	25.6	26.5	26.1	27.2
#831	25.2	27.4	27.3	27.3	30.4
Group 10					
#832	24.8	26.5	28.6	27.2	30.6
#833	25.0	27.0	28.1	28.0	32.0
#834	25.7	28.0	28.3	28.1	31.1
#835	23.8	26.7	27.3	27.2	31.8
#836	25.2	26.7	27.8	27.0	31.5
#837	28.2	30.9	31.9	31.4	34.5
#838	28.1	29.8	32.1	33.0	38.0
#839	27.6	30.6	30.8	30.5	32.8
Group 11					
#840	29.2	33.8	35.6	34.3	38.9
#841	25.3	28.1	29.1	29.4	34.1
#842	26.3	28.4	29.0	27.8	30.7
#843	26.9	27.9	30.1	29.8	30.4
#844	27.8	31.3	33.1	27.6	dead
#845	25.2	28.0	29.3	29.4	29.7
#846	26.3	27.4	30.7	29.7	30.0
#847	23.3	25.6	28.1	30.5	34.1
Group 12					
#848	25.0	27.3	29.6	31.6	34.4
#849	27.2	30.7	33.9	37.2	39.2
#850	26.1	30.1	35.9	36.1	42.0
#851	26.9	29.0	33.0	35.3	40.8
#852	27.9	32.4	37.2	38.5	37.7
#853	24.2	28.3	32.3	36.2	40.1
#854	24.5	28.5	32.3	34.4	37.3
#855	25.7	27.8	31.1	33.8	36.8

Table 7. Weights of turtles in Experiment 15, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 5					
#792	29.0	31.1	32.4	31.4	36.6
#793	27.0	28.7	29.2	29.6	34.2
#794	27.4	28.0	32.2	34.9	40.4
#795	23.8	24.9	27.1	30.3	dead
#796	26.7	29.9	28.9	28.3	29.6
#797	29.4	29.9	34.6	dead	dead
#798	30.8	32.1	34.8	34.7	38.9
#799	24.7	26.8	29.2	33.6	35.8
Group 6					
#800	23.7	25.8	26.9	25.2	25.7
#801	24.3	26.4	27.1	27.3	28.8
#802	24.2	26.5	25.9	24.8	24.8
#803	23.9	27.4	26.6	27.2	29.7
#804	25.6	27.9	29.4	28.4	32.8
#805	23.0	24.9	24.9	23.5	24.9
#806	27.0	28.2	30.8	33.0	32.4
#807	23.6	24.7	24.6	23.5	24.5
Group 7					
#808	24.6	26.5	27.5	29.1	30.6
#809	25.9	27.9	28.6	29.4	31.5
#810	29.2	31.6	32.3	33.0	39.1
#811	24.2	26.1	27.9	29.4	33.3
#812	22.7	25.4	26.7	27.3	29.1
#813	27.1	28.0	28.8	29.5	34.3
#814	25.9	27.9	28.5	28.1	29.1
#815	30.7	34.1	34.9	34.9	42.2
Group 8					
#816	24.3	25.9	27.1	27.1	30.4
#817	24.0	26.5	25.4	23.7	27.8
#818	26.0	27.7	29.1	29.5	32.0
#819	24.4	27.1	26.5	28.0	33.3
#820	24.5	26.4	26.8	26.5	28.8
#821	26.7	28.9	32.4	35.1	33.5
#822	25.3	27.9	28.8	28.5	31.7
#823	30.3	33.0	32.9	35.4	38.0

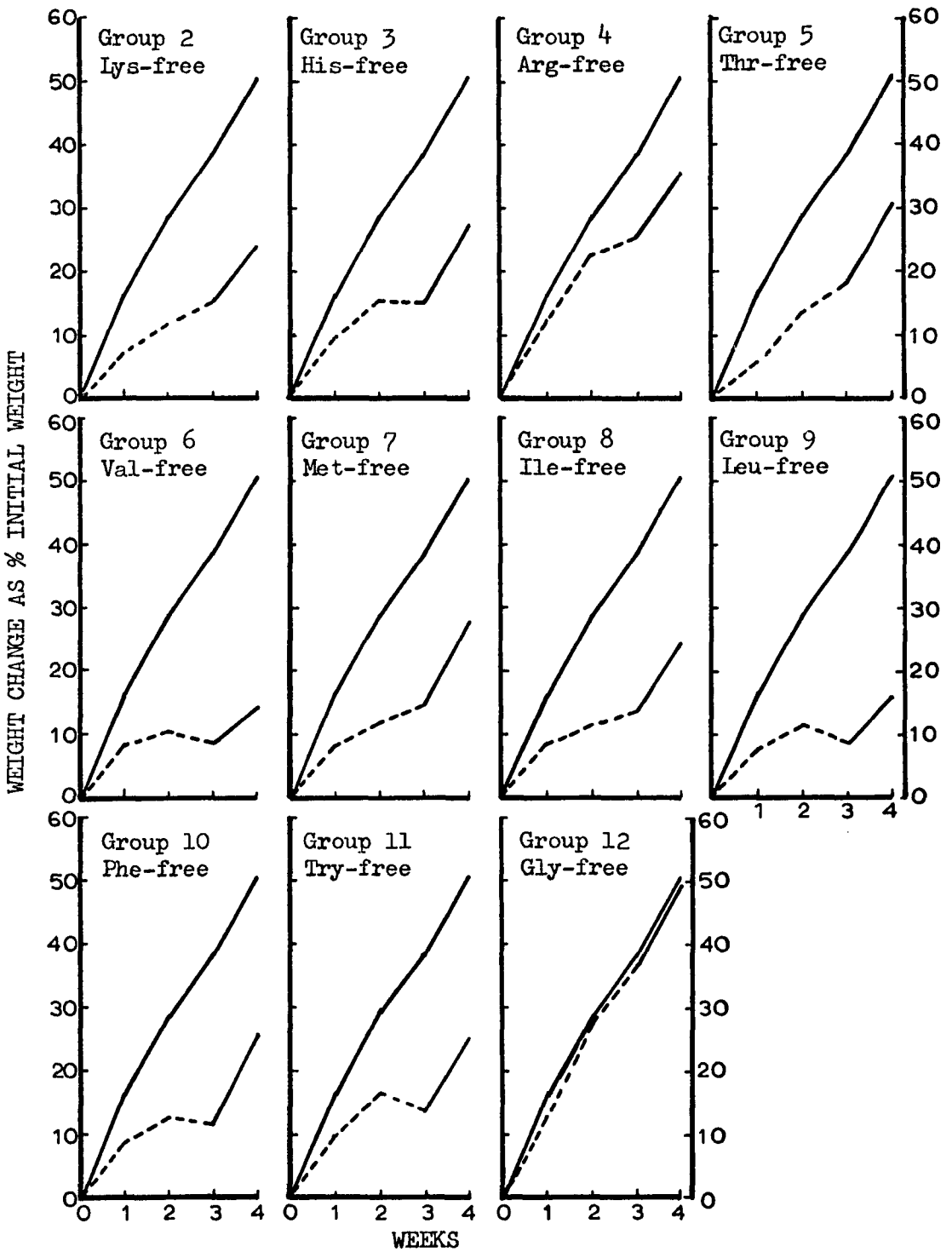


Figure 3. Average weight change of turtles in each group of Experiment 15 as a percentage of their initial weight. (———) Control Diet 121; (------) Deleted diet.

Table 8. Average percent of initial weight change values for each group of Experiment 15 at the end of the third week with subscript letter(s) for each group showing results of Duncan's multiple-range test. Groups with even a single subscript letter in common are not statistically significantly different at the P=.05 level

Group	Mean %	Subscript
Group 1	38.61	A
Group 12	36.52	A
Group 4	25.25	B
Group 5	18.18	BC
Group 3	15.24	C
Group 2	15.06	C
Group 7	14.70	C
Group 11	13.75	C
Group 8	13.46	C
Group 10	11.45	C
Group 9	8.85	C
Group 6	8.78	C

group fed the glycine-free diet (Group 12). There was, however, a significant difference between the control and all other groups. This again confirms that glycine is non-essential and that the remaining 10 test amino acids were of an essential or semi-essential nature. The L-arginine-free diet group (Group 4), as in the last experiment, occupied a more or less intermediate position. This group was statistically significantly different from all groups except Group 5 (L-threonine-free diet group) with a rate of growth less than the control or glycine-free diet group but with a rate of growth greater than that shown by Groups 2, 5, 6, 7, 8, 9, 10, or 11.

In summary, the results of this experiment matched closely with the results of Experiment 14. It was concluded that the nutritionally essential status of the amino acids L-histidine, L-threonine, L-valine, L-methionine, L-isoleucine, L-leucine, L-phenylalanine, L-lysine, and L-tryptophan were confirmed, with the amino acid L-arginine being shown to be nutritionally semi-essential.

CHAPTER 4

DISCUSSION

The results of this research show that methionine, phenylalanine, valine, isoleucine, leucine, threonine, tryptophan, histidine, lysine, and arginine are the essential amino acids required by the hatchling Green sea turtle, Chelonia mydas. The amino acids alanine, aspartic acid, glutamic acid, proline, serine, glycine, tyrosine, and cystine are non-essential for this turtle. In order to compare the qualitative amino acid requirement of the hatchling Green sea turtle with the requirements of other animal groups, the qualitative requirements for various mammals, birds, fish, and invertebrates are discussed below.

Amino Acid Requirements of Animals Other than the Sea Turtle

The qualitative amino acid requirements of the rat were determined over a 34 year period using purified and semi-purified diets. The essential amino acids required by the rat are tryptophan and lysine (Osborne and Mendel, 1914), histidine (Rose and Cox, 1924), phenylalanine (Womack and Rose, 1934), threonine (McCoy et al., 1935-1936), leucine and isoleucine (Womack and Rose, 1936), methionine (Womack, Kemmerer, and Rose, 1937), valine (Rose and Eppstein, 1939), and arginine (Borman et al., 1946). The non-essential amino acids are tyrosine (Womack and Rose, 1934), glycine and serine (McCoy and Rose, 1937), alanine (Gunther and Rose, 1938), aspartic acid (Rose and Fierke, 1942), cystine (Womack et al.,

1937), proline (Womack and Rose, 1947), and glutamic acid (Rose et al., 1948). The weanling pig was shown to have the same qualitative amino acid requirements as the rat (Mertz, Beeson, and Jackson, 1952). The essential amino acids required by man are given by Rose (1957) as valine, leucine, isoleucine, threonine, methionine, phenylaline, lysine, and tryptophan.

Early workers using purified diets demonstrated that the chick required tryptophan, arginine, and histidine (Klose, Stokstad, and Almquist, 1938), lysine (Almquist and Mecchi, 1942a), methionine (Grau and Almquist, 1943), phenylalanine, leucine, isoleucine, valine, threonine, and glutamic acid (Almquist and Grau, 1944), and glycine (Almquist and Grau, 1944; and Almquist et al., 1940). The non-essential amino acids were shown to be cystine (Grau and Almquist, 1943), tyrosine, alanine, aspartic acid, hydroxyproline, proline, and serine (Almquist and Grau, 1944). The role of glycine and its interrelationship with serine has been debated. Serine was claimed not to replace the need for glycine in the diet by Wixom et al. (1958). Baker, Sugahara, and Scott, 1968; Baker and Sugahara, 1970, showed that serine can completely replace the need for dietary glycine. Proline is required for optimum growth in the chick (Klain, Scott, and Johnson, 1959; Graber, Allen, and Scott, 1970; Roy and Bird, 1959; and Green, Scott, and Johnson, 1962). Halver (1957) developed a completely defined amino acid test diet for chinook salmon. The amino acids arginine, histidine, isoleucine, and valine were essential while the amino acids alanine, aspartic acid, glutamic acid, cystine, glycine, proline, hydroxyproline, serine, and tyrosine were non-essential

(Halver, DeLong, and Mertz, 1957). The same qualitative amino acid requirements have been found for the sockeye salmon (Halver and Shanks, 1960), rainbow trout (Shanks, Gahimer, and Halver, 1962), place and sole (Cowey et al., 1970), and channel catfish (Dupree and Halver, 1970).

The qualitative amino acid requirements of invertebrates are more variable (Table 9). The protozoans Trichomonas foetus and Tetrahymena geleii required the same 10 essential amino acids as the rat plus proline and serine for T. foetus and serine for T. geleii (Kidder and Dewey, 1945; Kidder and Dewey, 1951; and Weiss and Ball, 1947). The blowfly, Phormia regina, requires the same essential amino acids as the rat except that cystine can replace methionine and there is a requirement for either glutamic acid or aspartic acid (Hodgson, Cheldelin, and Newburgh, 1956; McGinnis, Newburgh, and Cheldelin, 1956; and Kastine and McGinnis, 1958). The larva of the mosquito, Aedes aegypti, has a requirement similar to the rat except the requirement for valine is questionable (Goldberg and de Meillon, 1948). The flour beetle Tribolium confusum, and the honey bee have qualitative amino acid requirements identical to the rat (Lemonde and Bernard, 1951; deGroot, 1953). The prawn, Palaemon serratus, has also been shown to have the same requirement as the rat (Cowey and Forster, 1971).

The qualitative amino acid requirements of the animals species discussed above are given in Table 9. Data is from references cited in text.

In general the qualitative requirements are remarkably similar with the invertebrates being somewhat more variable than the vertebrates. The amino acids leucine, isoleucine, tryptophane, methionine (except

Table 9. Amino acid requirements of various animal species

	Man	Young rat Weanling pig	Chick	Sea turtle C. mydas	Fish	Prawn	Blowfly	Honey bee	Tribolium confusum	Aedes aegypti larvae	Tetrahymena geleii	Trichomonas foetus
Valine	+	+	+	+	+	+	+	+	+	?	+	+
Leucine	+	∴	+	+	+	+	+	+	+	+	+	+
Isoleucine	+	+	+	+	+	+	+	+	+	+	+	+
Tryptophan	+	+	+	+	+	+	+	+	+	+	+	+
Methionine	+	+	+	+	+	+	+[a]	+	+	+	+	+
Threonine	+	+	+	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	+	+	+	+	+	+	+
Histidine	-	+[b]	+	+[b]	+	+	+	+	+	+	+	+
Arginine	-	+	+[c]	+	+	+	+	+	+	+	+	+
Glycine	-	-	+	-	-	-	-[a]	-	-	+	-	+
Cystine	-	-	-	-	-	-	+	-	-	-	-	-
Alanine	-	-	-	-	-	-	-[d]	-	-	-	-	-
Aspartic acid	-	-	-[b]	-	-	-	+[d]	-	-	-	-	-
Glutamic acid	-	-	+[b]	-	-	-	+	-	-	-	-	-
Proline	-	-	+	-	-	-	+	-	-	-	-	+
Tyrosine	-	-	-[c]	-	-	-	-	-	-	-	-[e]	-
Serine	-	-	+	-	-	-	-	-	-	-	+	+

[a]either methionine or cystine is required; [b]required for maximum growth; [c]either glycine or serine is required; [d]either aspartic acid or glutamic acid is required; and [e]serine requirement depends upon strain.

possibly the blowfly), threonine, lysine, phenylalanine, and valine (except possibly the larval mosquito) are required by all animals tested. Histidine and arginine have not been shown to be essential for man but they are required by all other animals discussed at least for maximum growth.

It is interesting to consider phylogenetic relationships in terms of qualitative amino acid requirement between reptiles and mammals, birds, and fish. Both mammals and birds evolved from reptilian ancestors. Reptiles evolved from amphibian ancestors, and the amphibian ancestors evolved from primitive fish (Romer, 1960; Swinton, 1960).

In general the amino acid requirements of the four classes are very similar with the exception of the arginine requirement and the chicks requirement of glutamic acid, proline, and glycine or serine for maximum growth. The glycine or serine requirement in birds is apparently a result of the high demand for glycine in feather formation (Hegsted et al., 1941). The growth-promoting effect of proline and glutamic acid under certain conditions for chicks has been documented but not explained.

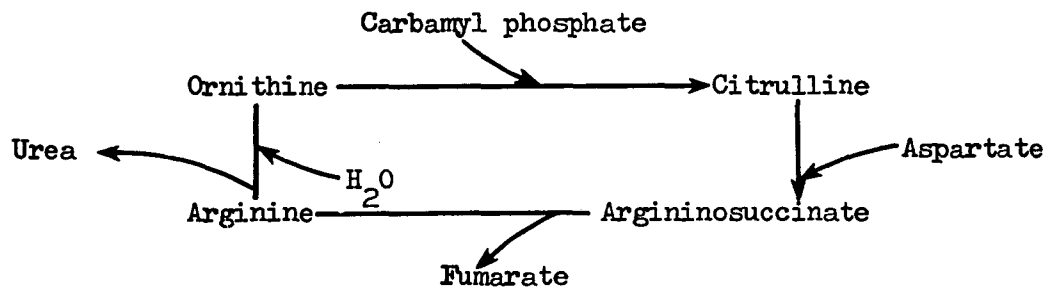
The Green sea turtle actually has a qualitative amino acid requirement more similar to that of the young rat or weanling pig than to birds or fish when the effects of complete arginine deletion from the diet are considered. Both the bird and fish cannot gain weight on diets lacking arginine while complete removal of this amino acid from diets fed the hatchling Green sea turtle or the young rat or pig results in a decrease in the rate, but not cessation of growth. The qualitative amino acid requirement of the Green sea turtle, then, offers little in

the way of surprise, with the required amino acids being consistent with those required by other vertebrates.

Discussion of Arginine Requirement

As mentioned above, the hatchling sea turtle, young rat, and weanling pig are all capable of reduced growth on a diet completely lacking in arginine while the chick and teleost fishes lose weight on such diets. This indicates that the hatchling sea turtle, young rat, and weanling pig have a greater ability to synthesize this amino acid than do chicks or fish.

Synthesis of arginine involves the Krebs urea cycle given below (Meister, 1965; Harper, 1971).



For there to be a net synthesis of arginine, arginine produced must not be cleaved to form urea and ornithine, and at some point in the cycle, an intermediate must be synthesized from non-cycle precursors.

It is tempting to relate the varying abilities to synthesize arginine in the animals discussed to the presence or absence of the Krebs urea cycle.

Teleost fish are ammonotelic and cannot synthesize urea via the Krebs ornithine-citrulline-arginine cycle (Forster and Goldstein, 1969). The absence of the urea cycle would explain the inability of teleosts

to synthesize arginine and the subsequent growth failure on diets devoid of this amino acid.

In the chick, ornithine cannot but citrulline can efficiently replace dietary arginine, with the chick lacking the mechanism or arginine formation and decomposition which exists in the mammal (Klose et al., 1938; Klose and Almquist, 1940). Meister (1965) states that the enzyme carbamyl phosphate synthetase, which is responsible for catalyzing the formation of carbamyl phosphate from carbon dioxide, ammonia, and phosphate, is not present in the chick kidney or liver, with evidence that the chick cannot synthesize ornithine nor can it convert ornithine to citrulline. Thus, the inability of the chick to grow on diets lacking arginine can be explained.

As has been discussed previously, mammals either require no arginine, as in the case of man, or arginine is required only during the period of rapid growth, as in the young rat and weanling pig. The mammalian liver and kidney both have large capacities to synthesize arginine and citrulline, with the majority of plasma arginine arising from the kidney, which exhibits relatively little arginase activity as compared to the liver (Meister, 1965).

The problem now arises of accounting for the apparent relatively high rate of synthesis of arginine by the Green sea turtle. The difficulty is caused by confusion in the literature as to what the major nitrogenous excretory product is in the urine of the hatchling Green sea turtle. Khalil (1947) reports that ammonia is the major excretory product with only one turtle of the four tested having any urea in the urine although all animals had high levels of urea in the blood plasma and low

plasma ammonia levels. Moyle (1949), in discussing nitrogenous excretion in Chelonian reptiles, states that Khalil's report contradicts Lewis (1918) who found Chelonia mydas to excrete primarily urea. It is difficult to judge the validity of this statement since the paper by Lewis fails to make any mention of the species of turtle tested. Moyle, who may have had particular knowledge, says it was C. mydas. Results of my feeding diets completely lacking arginine show that the hatchling Green sea turtle can synthesize a portion of its arginine requirement. This suggests the presence of a developed urea cycle. This is supported to some extent by Khalil's (1947) findings of high plasma urea values in this species.

Glycine Tolerance

In Experiment 13, Group 4 was fed a diet containing 18% amino acid mixture plus 11.4% glycine as a nitrogen supplement. In this experiment, glycine had a positive growth-promoting effect equaling the growth response obtained on an equal amount of amino acid mixture. This positive effect of feeding a high level of glycine to hatchling sea turtles is somewhat surprising in light of the effect of high levels of glycine on chicks. Glycine was essential for growth in the chick but glycine at levels of 2% or more was toxic and depressed the growth rates (Almquist and Mecchi, 1942b). The role of vitamins in glycine toxicity has been studied repeatedly. The nicotinic acid requirement for chicks increased to as much as 5 mg/100 g of diets containing combinations of arginine, glycine, and alanine (Briggs, Groschke, and Littie, 1946). Folacin at 10 mg/100 g of diet prevented glycine toxicity in

chicks fed diets containing 5% glycine but folacin had no effect on toxicities caused by 5% levels of tyrosine or alanine (Naber et al., 1956). Kratzer and Lantz (1957), suggest that glycine is toxic by causing a folic acid deficiency in turkey poults. Vitamin B_{12} and folic acid were shown to counteract toxicity in chickens resulting from 3, 6, and 9% levels of glycine. Vitamin B_{12} was more effective than folic acid (Machlin et al., 1952).

The apparent high tolerance of glycine demonstrated in Experiment 13 is difficult to explain. The most obvious area to consider is the level of folic acid and niacin in the diet fed the turtles. The level of folic acid used in the turtle diets was 0.2 mg/100 g of diet as compared to the 10 mg/100 g of diet required to prevent glycine toxicity in the chick. The level of niacin in the turtle diet was 11 mg/100 g of diet, more than twice the level required to prevent toxicity symptoms in the chick. The difference in effect of high levels of glycine on chicks and the hatchling sea turtles merits further research, although the level of folic acid does not seem to be responsible for the tolerance to glycine shown by the sea turtle.

Practical Applications of Results and Suggested Future Research

Knowledge of the qualitative amino acid requirements of the hatchling Green sea turtle provides the groundwork for future research on the amino acid requirements of the sea turtle. Next, the amino acid requirements should be determined quantitatively. Research is also needed on required levels of protein, efficiency of utilization of various carbohydrates, lipid requirements, and vitamin requirements. As

this information becomes available, prepared rations can be formulated on a more factual basis, using the least expensive ingredients to fulfill the requirements. The objective would be to utilize the protein fed for protein synthethis and to obtain energy for metabolic functions from the cheaper fat and carbohydrate sources.

One area of research which should be investigated is the development of a method for the collection of total excreta from turtles during feeding trials. Once such a technique is developed, many standard nutritional techniques and tests can be performed providing information on digestibility and retention of food stuffs as well as providing the means of conducting nitrogen balance studies.

APPENDIX A

DETAILS OF METHODS, MATERIALS, AND RESULTS OF EXPERIMENTS 1-12 WITH ALL ILLUSTRATIONS AND TABLES

Experiment 1

Objective

The purpose was to determine if the amino acids L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, and glycine were essential.

Materials and Methods

Hatchling Green sea turtles (Chelonia mydas) used in this experiment were obtained from Heron Island, Australia. Upon arrival the hatchlings were placed in artificial sea water (35°/oo, 26°C.). The following day all turtles were individually weighed and a number was placed on the plastron with a felt-tipped marker. Of the 181 hatchlings received, approximately one-third had what appeared to be a fungal infection. Since only 128 were needed for the experimental groups, the 53 excess turtles were removed by actively selecting against the more severe fungal infections and against abnormal numbers of laminae on the carapace. This resulted in some of the hatchlings used in the experimental groups having slight fungal infections. The hatchlings were randomly divided into 8 groups, each containing 16 turtles. The total and average weight for each group was recorded.

Each group was confined in a plastic tub measuring approximately 90 cm in length and 40 cm in width with an average water depth of 10 cm. Water was circulated and temperature was controled with an army surplus photographic temperature control unit designed for the temperature regulation of chemical solutions. This unit consisted of a heating element, a refrigeration unit, and a pump. The pump forced water at the rate of approximately 3700 liters per hour to a 75 liter plastic container

setting above the tubs. Gravity then carried water from this container by 8 plastic hoses to the tubs. Water from the tubs was carried to a second 75 liter plastic container on the floor which received the intake hose for the heating-cooling unit pump as well as the intake and output hoses of a distomaceous earth filter which provided a continuous filtration rate of 4500 liters per hour.

The room in which the hatchlings were kept had only a double sheet of plastic as a roof. Room temperature was maintained with the aid of a small electric heater. It was expected that water temperature could be maintained between 24-30°C. Water temperature as low as 21°C. was recorded during the 2 weeks of the experiment.

During the experiment hatchlings were kept in salt water made by mixing Morton's water softener salt with tap water. Salinity as determined by an American Optic T/C refractometer was 29°/oo. The water was changed at the end of each week and the system cleaned. The filter was cleaned two or three times each week.

The amount of amino acid mixture, carbohydrate, and fat in the purified diet used in this experiment was based on the composition of shrimp as given by the United States Department of Agriculture (1963) since both Harrisson (1955) and Caldwell (1962) had reported raising hatchlings for up to three years on a diet of shrimp. The amino acid mixture was formulated to contain the 18 common amino acids in the proportion they occur in shrimp as reported by Brogstrom (1962).

Group 1 received the complete, defined synthetic diet (Diet 001) containing all 18 amino acids to be tested during the course of this

research program. Groups 2, 3, 4, 5, 6, and 7 each received diets similar to Diet 001, except in each case a different single amino acid was deleted and replaced by an equal weight of ammonium bicarbonate. The purpose of the ammonium bicarbonate was to reduce the acidity of the diets resulting from the high levels of free amino acids. Group 2 received a glycine-free diet (Diet 002), Group 3 received an L-alanine-free diet (Diet 003), Group 4 was fed an L-valine-free diet (Diet 004), Group 5 received an L-leucine-free diet (Diet 005), Group 6 received an L-isoleucine-free diet (Diet 006), and Group 7 was fed an L-serine-free diet (Diet 007). Group 8 was fed a diet of chopped shrimp (Diet 008) and was to serve as a disease control as well as to provide growth values to which growth obtained on Diet 001 could be compared. The composition of Diet 001 is given in Table 1A. Tables 2A and 3A give the composition of the Vitamin Diet Fortification Mixture and composition of Hawk Oser Salt Mixture No. 3 used in the diets. After the end of the first week, all of the purified diets were modified by adding 0.5 grams of ammonium bicarbonate to each of the 7 diets to provide additional neutralization of the free amino acids.

The diets were prepared by combining the agar and water and then heating this mixture to 90°C. while stirring. The mixture was then allowed to cool to 55°C., at which time the remaining dietary ingredients were added and thoroughly mixed. While still fluid, the diet was poured into labeled 100 mm petri dishes and placed in a refrigerator for storage. Upon cooling, the diet was very firm and capable of being cut into blocks for feeding.

Table 1A. Chemical composition of Diet 001

Component	gm/100 gm of diet
L-Lysine . HCl	1.70
L-Histidine · HCl · H_2O	.34
L-Arginine · HCl	1.63
L-Aspartic Acid	2.12
L-Threonine	.74
L-Serine	.76
L-Glutamic Acid	3.17
L-Proline	.67
Glycine	.85
L-Cystine	.20
L-Valine	.80
L-Methionine	.51
L-Isoleucine	.69
L-Leucine	1.56
L-Tyrosine	.74
L-Phenylalanine	.80
L-Tryptophan	.18
L-Alanine	1.09
Amino Acid Total	18.55
Dextrose	1.50
Vitamin Diet Fortification Mixture	2.50
Hawk Oser Salt Mixture No. 3	1.40
Corn oil	2.00
Agar	3.00
Distilled Water	71.05

Table 2A. Chemical composition of Hawk Oser Salt Mixture No. 3

Compound	Percent
Calcium carbonate	6.86
Calcium citrate	30.83
Calcium biphosphate	11.28
Magnesium carbonate	3.52
Magnesium sulfate	3.83
Potassium chloride	12.47
Dibasic potassium phosphate	21.88
Sodium chloride	7.71
Cupric sulfate	.0078
Ferric ammonium citrate	1.528
Manganese sulfate	.0201
Ammonium alum	.00923
Potassium iodide	.000015
Sodium fluoride	.05071

Table 3A. Chemical composition of Vitamin Diet Fortification Mixture

Compound	Percent
Vitamin A concentrate	.45
Vitamin D concentrate	.025
Alpha tocopherol	.50
Ascorbic acid	4.5
Inositol	.50
Choline chloride	7.5
Menadione	.225
p-Aminobenzoic acid	.50
Niacin	.45
Riboflavin	.10
Pyrodoxine hydrochloride	.10
Thiamine hydrochloride	.10
Calcium pantothenate	.30
Biotin	.002
Folic acid	.009
Vitamin B-12	.000135
Dextrose	84.604

The hatchlings were fed twice each day for approximately 30 minutes each period for the first 2 days of the experiment. On the third day this procedure was modified in an effort to increase feeding. This modification consisted to allowing uneaten food from the morning feeding to remain in the tubs until the evening feeding and, conversely, by allowing uneaten food from the evening feeding to remain in the tubs over-night until the following morning. A record was kept of the amount of diet fed each group. Uneaten food was removed, blotted dry, and weighed and this value recorded. The amount ingested by each group was determined by subtracting the uneaten food weight from the amount of food fed. The hatchlings were fed 7 days a week and average weight of hatchlings of each group was determined at the beginning of each week by weighing the total group and dividing by the number of hatchlings in the group.

The skin infections were treated at the time of each weighing by letting the hatchlings dry and then topically applying merthiolate to diseased areas.

Results and Interpretation

The hatchlings ate little during the first 2 days of the experiment. On the third day all the groups except Group 1 began to feed fairly well, ingesting an average of about 9 grams/day/group. For the next 5 days Groups 2-7 ate approximately 20 grams/day/group, while during the same 5 day period Group 1 ingested an average of only 6 grams/day and Group 8 ingested slightly more than 30 grams/day. Since ingestion of the deleted synthetic diets was better than the amount

consumed of the complete diet, it was felt that perhaps the absence of ammonium bicarbonate in Diet 001 was causing the acidity of this diet to be too high and making this diet unpalatable. To correct for this, after the first week 0.5 grams of ammonium bicarbonate was added to all the synthetic diets, thereby reducing the acidity of Diet 001 and maintaining the approximate isonitrogenous nature of diets 002-007.

At the beginning of the second week, feeding decreased in all of the synthetic diet groups to an average of 4 grams/group/day. During this same period, Group 8 had an average intake of 33 grams/day.

The average weight of hatchlings in each group is given for each week of the experiment in Table 4. Note that all groups gained weight during the first week with Group 8 (shrimp diet) gaining the greatest amount. In each group, much of this growth was due to the utilization of stored yolk. During the second week all groups fed the synthetic diets lost weight while the shrimp-fed group showed only a slight increase.

Deaths began to occur in the experimental groups on the eleventh day with 5 hatchlings dying. At the end of the second week, 3 additional hatchlings had died. No cause could be determined for the deaths and the deaths were fairly evenly distributed among the groups with only Group 1 having lost no hatchlings and Group 3 having lost 2 turtles. The remaining 6 groups had lost 1 hatchling each.

The experiment was originally planned to last 4 weeks, but during the first 5 days of the third week 30 hatchlings died. It was terminated at this point. Results obtained indicated that the test synthetic diet as fed was not suitable.

Table 4A. Average weight in grams of turtles in each group of Experiment 1 during experimental period

Week	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
0	24.1	24.6	24.0	24.2	24.0	24.1	24.4	23.9
1	26.8	28.8	28.2	28.1	28.5	27.8	27.9	31.2
2	26.1	27.0	26.7	26.5	26.5	25.7	25.9	32.1

Experiment 2

Objective

The purpose of this experiment was to test the effectiveness of 3 different pH and protein levels in making the synthetic diet suitable for the hatchling Green sea turtle.

Materials and Methods

The turtles used in this experiment were the survivors of the groups which received the synthetic diets in Experiment 1. After the termination of Experiment 1 these turtles were fed chopped shrimp during the 17 days between experiments. The hatchlings were randomly divided into 9 groups, each containing 5 turtles. Total and average weight was determined for each group the morning the experiment was begun. Average weights were to be determined for each at the beginning of each week.

The water system was the same as in Experiment 1 except an additional tub was added for the ninth group.

The diets received by the nine groups varied in both pH and amino acid level. The amino acid levels tested were to be 9%, 18%, and 36% of the wet weight of the diets and each level was to be tested at a pH of 3, 5, or 7. The pH of the diets was obtained by dissolving the amino acid mixture in distilled water and then using a pH meter to monitor pH level while the pH was raised or lowered to the level desired by using dilute sodium hydroxide or hydrochloric acid, respectively. It was not possible to obtain a sufficiently fluid consistency for the 18% and 36% amino acid levels without adding additional distilled water. The modification of pH also required addition of fluid, and both these factors resulted in the amino acid level of all diets being slightly below intended levels. The composition of these test diets was basically as shown in Table 1A except that, as the level of the amino acid component was varied, a corresponding inverse change in the amount of distilled water was necessitated. The relative proportions of dietary ingredients other than amino acid mixture and water remained as in Experiment 1.

The groups received the following: Group 1, a 8.6% amino acid diet at pH 3 (Diet 009); Group 2, a 16.2% amino acid diet at pH 3 (Diet 010); Group 3, a 29.2% amino acid diet at pH 3 (Diet 011); Group 4, a 8.3% amino acid diet at pH 5 (Diet 012); Group 5, a 16.8% amino acid diet at pH 5 (Diet 012); Group 6, a 29.0% amino acid diet at pH 5 (Diet 013); Group 7, a 7.9% amino acid diet at pH 7 (Diet 014); Group 8, a 14.9% amino acid diet at pH 7 (Diet 015); and Group 9, a 25.9% amino acid diet at pH 7 (Diet 016).

The diets were prepared as in Experiment 1 except, as already noted, the amino acid mixture and distilled water were mixed together first to adjust the pH of the various diets. The feeding procedure and diet storage was the same as in the previous experiment.

Results and Interpretation

The relative amounts of diet consumed by each group is shown in Figure 1A. As pH increased, the effect of amino acid level on diet consumption was greatly magnified. At pH 3 little difference existed between the diet consumption at the 3 amino acid levels. At pH 5 less of the low amino acid level diet was ingested than at pH 3; the medium level protein diet was ingested at about the same level as at pH 3; and, the high amino acid level diet was ingested at about twice the rate as at pH 3. At pH 7 the low amino acid level diet was consumed at its lowest rate, consumption of the medium amino acid diet increased slightly, and the high amino acid ration was ingested in extremely large amounts. It is possible that the food intake value given for Group 4 is too low, since one turtle in this group was dead on the morning of the seventh day. If this turtle was diseased, then its appetite may have been effected and the remaining 4 turtles would have ingested a greater percentage of their initial weight. The results indicated the possibility that the palatability of the diet could be improved by increasing the level of protein and raising the pH above the normal test diet level of pH 5.

The average initial weight and the weight at the end of the first week for the turtles of each group are shown in Table 5A. As can

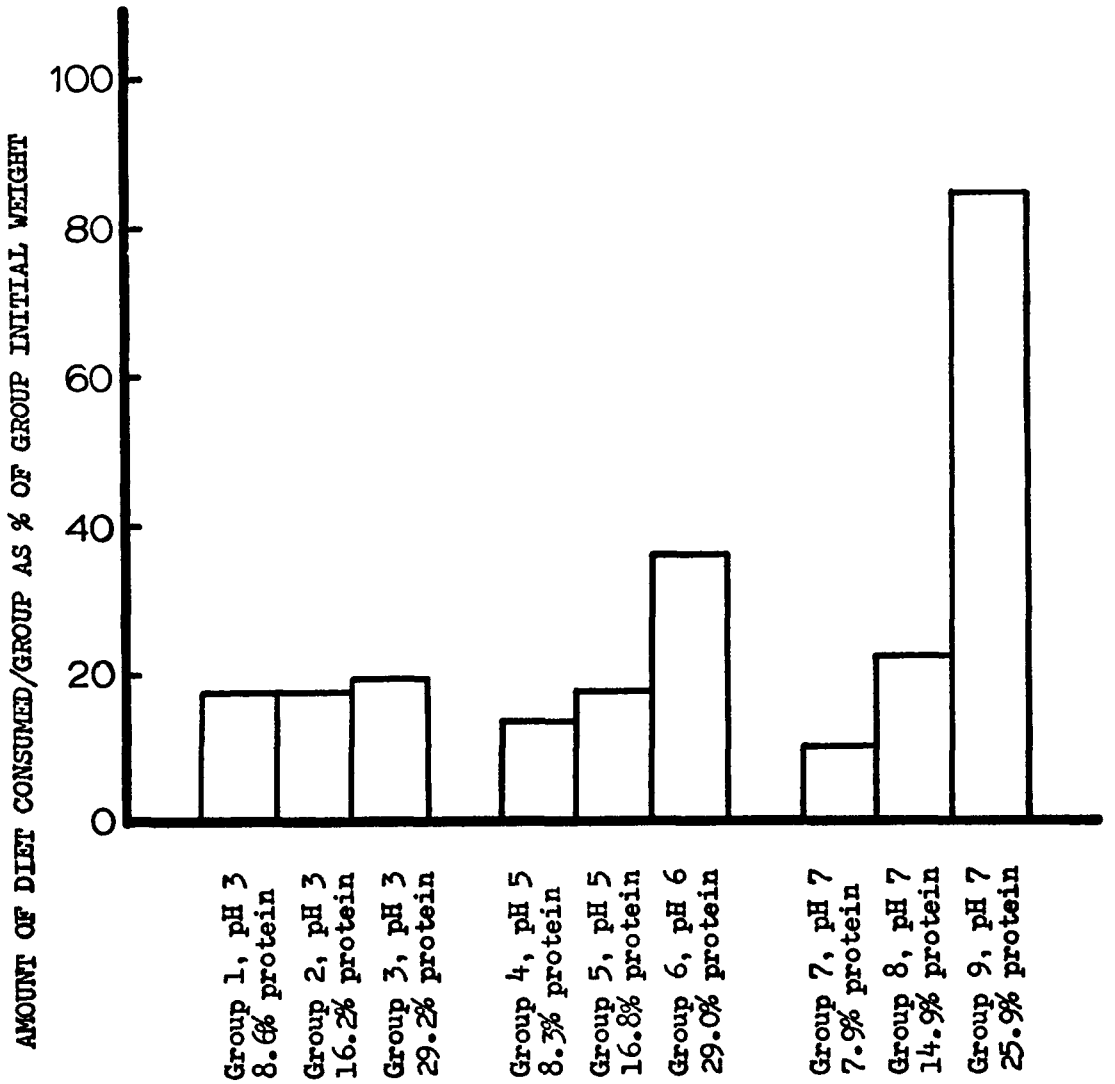


Figure 1A. Amount of diet consumed for each group of Experiment 2 expressed as a percentage of group initial weight

Table 5A. Average weight in grams of turtles in each group of Experiment 2 during experimental period

Week	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
0	48.0	42.2	43.4	42.6	45.6	48.4	43.0	44.4	44.8
1	44.4	37.8	39.4	40.5	42.4	47.0	40.0	41.0	43.0

be seen from this table, all groups lost weight during the week. The percentage of initial weight lost ranged from 2.9% for Group 6 to 10.4% for Group 2. Weight lost seemed to be relatively independent of diet protein level or pH. The fact that Group 9, after having ingested such a large amount of diet, still lost weight strongly suggests that the fault was with the composition of the diet and not with its palatability. It was possible that the diets did not contain sufficient energy to promote growth, especially if the amino acids were leaching from the diets in any quantity.

Experiment 3

Objective

The objectives of this experiment were: (1) to determine if increasing the amount of dextrose in the complete diet used in Experiments 1 and 2 would result in the diet promoting growth in hatchlings, and (2) to test the growth-promoting effectiveness of a purified diet using vitamin free casein as the protein source along with a high dextrose level.

Materials and Methods

Two different groups of turtles were used in this experiment. One group consisted of C. mydas hatchlings provided by Dr. Archie Carr, University of Florida. The other group consisted of 4 month-old C. mydas turtles which were survivors of the first 2 experiments. A brief history of each group is given below.

The 12 hatchlings from Dr. Carr were from the Ascension Island population. Upon arrival in Tucson, the hatchlings were kept in artificial sea water (Rila brand) at a salinity of 35°/oo. The container used was a tub as described in the first experiment. Water temperature was ambient room temperature (20-23°C.). The hatchlings were fed chopped shrimp twice each day. Only one turtle had any evidence of skin infection and this was treated using merthiolate as well as by placing all hatchlings in a 2 ppm solution of methylene blue for 2 two-day intervals. One hatchling died 12 days after arrival, but no cause of death could be determined. Fifteen days after their arrival the hatchlings were transported in a styrofoam container with wet paper towels to the University of Arizona marine lab at Puerto Penasco, Sonora, Mexico. Upon arrival the hatchlings were placed in a tub with running sea water until the experiment was begun 4 days later.

The second group of turtles had been maintained on shrimp at the Tucson lab. These turtles were transferred to Mexico at the same time as the smaller hatchlings and were maintained in tubs with running sea water until the experiment was begun.

The 9 healthiest Ascension Island hatchlings were randomly divided into 3 groups of 3 hatchlings each. Group 1 received a diet of chopped shrimp (Diet 018). Group 2 received a complete amino acid diet (Diet 019) similar to Diet 001 fed in Experiment 1 except that the amino acid level of Diet 019 was 18.0% and the dextrose level was 20.0%. Group 3 received a synthetic diet (Diet 020) which contained vitamin-free casein as the protein source. This diet required more water than Diet 019 to achieve a fluid consistency; therefore, the planned casein level of 18% as well as the level of all other dietary ingredients were decreased. The composition of this diet is given in Table 6A.

Table 6A. Chemical composition of Diet 020

Component	gm/100 gm of diet
Vitamin Free Casein	14.4
Dextrose	16.0
Vitamin Diet Fortification Mixture	2.0
Hawk Oser Salt Mixture No. 3	1.1
Corn Oil	1.6
Agar	2.4
Distilled Water	62.5

The 17 4-month old turtles from Tucson were placed in 3 groups. Group 4 contained 5 turtles and received a diet of chopped shrimp (Diet 018). Group 5 contained 6 turtles and received Diet 019. Group 6 contained 6 turtles and received Diet 020.

The turtles in each group were individually weighed and had numbers painted on their plastrons with a felt-tipped pen. The turtles were fed 7 days a week, twice each day with a morning feeding and an evening feeding. A record was kept of the amount consumed by each group. The turtles were individually weighed on the 6th, 14th, 21st, and 28th days of the experiment and turtles with skin infections received topical applications of merthiolate at the time of weighing. Diet preparation, feeding procedure, and diet storage were the same as in Experiment 1.

The turtles were kept during the experiment in a partially covered, ground-level cement pit. This pit was approximately 6.1 m x 3.0 m x 2.4 m. The floor sloped to a center drain. A standpipe maintained the water level at about 75 cm. Water flowed into the pit at approximately 400 liters/min. and a temperature of 29°C. was maintained within 2°C. by mixing hot and cold sea water before it entered the pit. Turtles of each group were contained in pens 48 cm. x 107 cm. x 38 cm. consisting of a wooden frame with sides and bottom of plastic mosquito screening. The containers were suspended so that water depth was approximately 28 cm. Salinity was constant at 34°/oo.

Results and Interpretations

The weights of each hatchling during the 4 weeks of the experiment are given in Table 7A. This table shows that the average initial weight of hatchlings in Groups 1, 2, and 3 varied by less than a gram while the average initial weight of turtles in Groups 4 and 6 are very

Table 7A. Individual weights in grams of turtles in each group of Experiment 3 during experimental period

Group	Initial	6 days	14 days	21 days	28 days
Group 1					
# 4	28.7	36.8	dead	dead	dead
# 6	37.5	52.2	74.8	95.9	116.0
# 7	38.4	dead	dead	dead	dead
Group 2					
# 1	33.7	39.8	47.4	47.6	46.0
# 5	36.6	40.8	49.6	52.1	41.2
# 8	34.0	37.3	40.7	40.3	38.6
Group 3					
# 2	33.3	42.5	53.0	58.2	53.1
# 3	36.6	43.9	52.8	62.8	69.6
# 9	36.5	44.7	56.9	64.6	73.1
Group 4					
#10	132.7	150.2	166.4	178.0	187.2
#14	126.7	143.2	162.3	170.0	174.7
#18	100.0	118.1	147.4	139.8	148.3
#19	97.2	114.0	127.6	112.5	121.6
#25	73.4	92.0	97.2	100.0	100.4
Group 5					
#12	96.0	95.2	91.5	106.6	104.9
#13	101.5	89.0	88.3	93.2	96.8
#17	90.2	89.0	94.6	87.8	dead
#20	78.8	86.8	82.3	82.4	83.9
#22	109.9	107.6	102.6	82.1	dead
#26	83.3	85.7	81.7	77.2	72.9
Group 6					
#11	96.5	101.9	116.1	126.1	128.9
#15	130.7	132.5	122.4	114.2	dead
#16	96.8	124.6	139.3	158.7	167.1
#21	115.5	106.0	91.4	dead	dead
#23	110.3	107.8	121.0	128.7	134.6
#24	97.1	92.6	95.6	107.2	114.7

similar but approximately 13 grams above that of turtles in Group 5. Table 7A also gives information on mortality within each group.

Figure 2A gives the average weight change of each turtle as a percentage of its initial weight for each period between weighings. Group 1 had the most rapid rate of weight gain and, even though this group is represented for the most part by the growth of a single turtle, it demonstrates the rapid growth possible by this species when fed a suitable diet, such as shrimp. Comparison of Groups 2 and 3 shows the vitamin-free casein to be a more acceptable protein source than the amino acid mixture used when growth is the criterion; however from Figure 3A, which shows the rate of food consumption for each group, it can be seen that more of Diet 019 was ingested by Group 2 than Diet 020 by Group 3.

Growth response by the larger turtles in Groups 4, 5, and 6 was similar to that of the other 3 groups in that the shrimp-fed group had the greatest rate of growth followed very closely by Group 6 fed on Diet 020 and with Group 5 gaining the least--in fact losing weight.

It can be noted from Figure 3A that both Groups 2 and 5 ingested maximum amounts of Diet 019 during the second week and then the amount consumed dropped rapidly the remainder of the experiment. This was interpreted to indicate that Diet 019 became less palatable after the initial two weeks, although the additional dextrose seemed to slightly improve the growth-promoting properties of the diet.

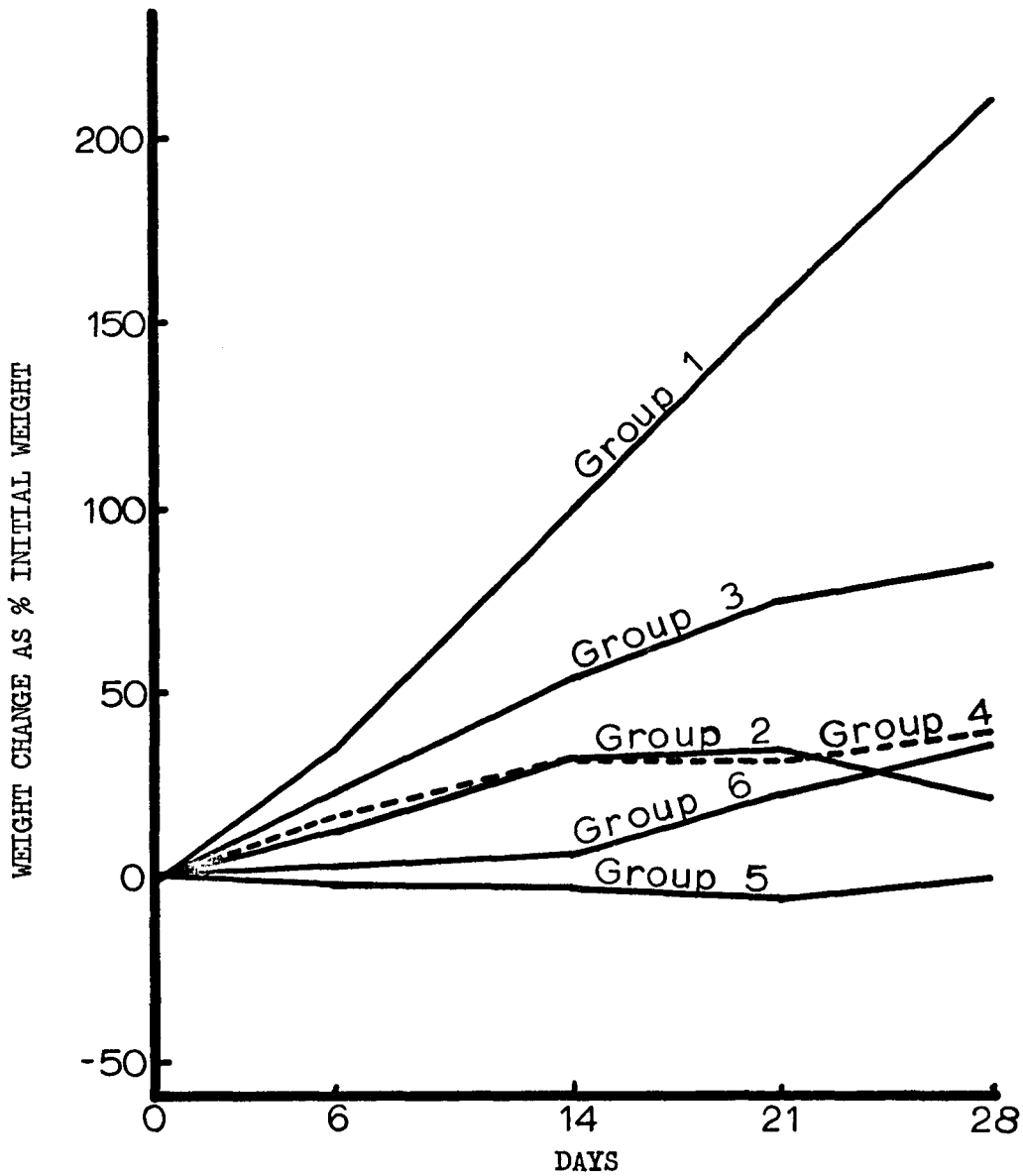


Figure 2A. Average weight change of turtles in each group of Experiment 3 as a percentage of their initial weight

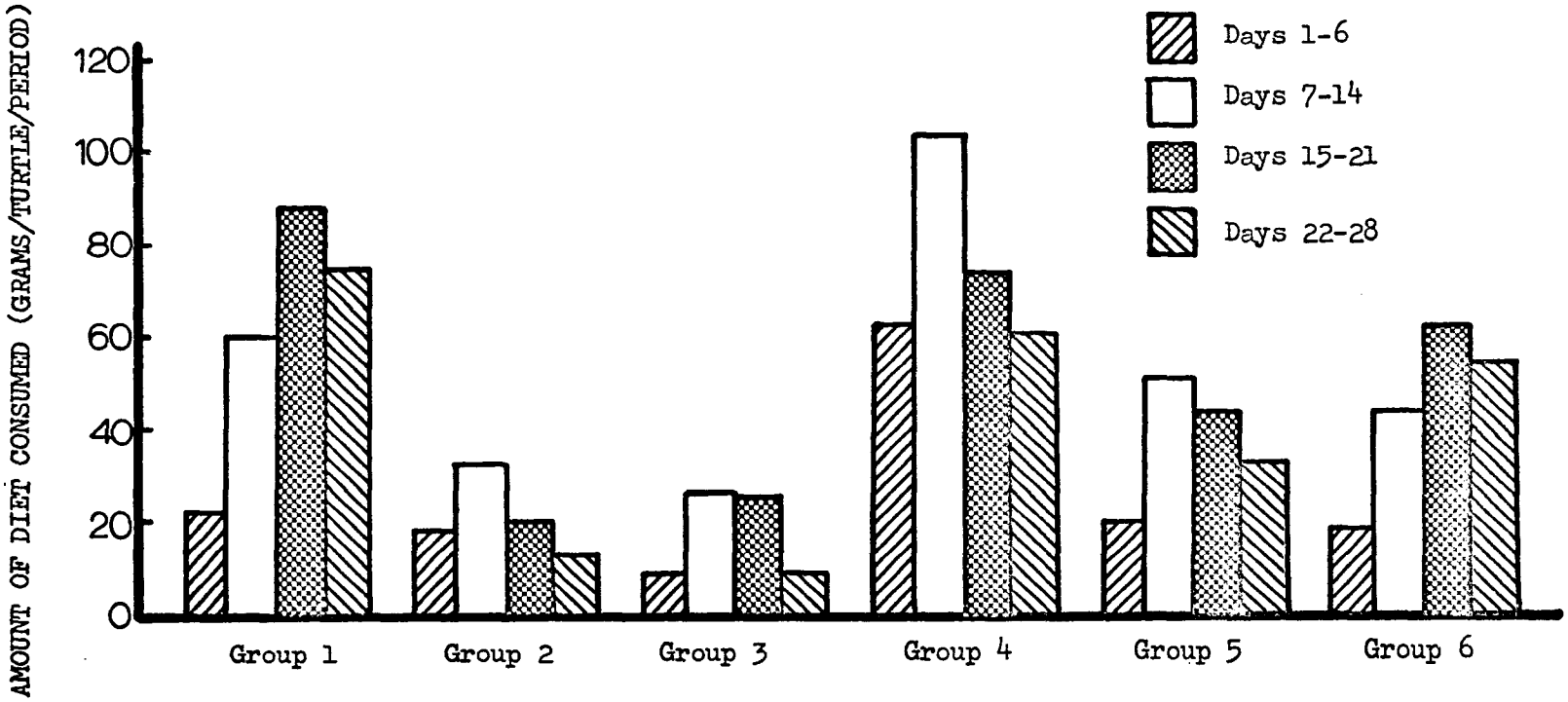


Figure 3A. Food consumption between weighings for turtles in Groups 1-6 of Experiment 3

Experiment 4

Objectives

This experiment was to determine: (1) if increasing the amount of dextrose from 20% to 30% of the diet (wet weight) would improve the growth-promoting properties of both the amino acid diet and the casein diet, (2) if the addition of sodium bicarbonate would enhance the palatability of the amino acid diet, and (3) if decreasing the amount of casein would decrease growth response.

Materials and Methods

The turtles used in this experiment were survivors of Groups 2, 3, 5, and 6 of Experiment 3. These turtles had been fed chopped shrimp during the 10 days prior to beginning this experiment and all turtles had gained weight during this period. Group 1 contained the 4 surviving turtles of Groups 2 and 3. The 8 remaining turtles of Groups 5 and 6 were randomly divided into 2 groups each containing 4 turtles (Groups 2 and 3). The turtles of each group were individually weighed at the beginning of the experiment and at the start of each following week.

Group 1 received Diet 021 which was similar to Diet 019 except the amount of dextrose had been increased to 30% wet weight of diet with a corresponding decrease in water content and sodium bicarbonate was added at the 2% level. This diet was modified 2 days after beginning the experiment by decreasing the amount of sodium bicarbonate to 1% and raising the level of agar from 3% to 4% since this diet tended to dissolve more rapidly than previous diets making it difficult to

determine the amount of diet ingested. Group 2 received Diet 022 containing 7.2% vitamin-free casein and 24% dextrose. Group 3 received Diet 023 containing 14.4% vitamin-free casein and 24% dextrose. The composition of Diets 021, 022, and 023 is given in Table 8A.

Diet preparation, feeding procedure, and diet storage was the same as in Experiment 3. One difference in experimental procedure between Experiment 3 and this experiment involves the treatment of skin infections. Group 1 was treated twice weekly with topical applications of 1% gentian violet in 95% ethanol, while turtles of Groups 2 and 3 were treated twice weekly with topical applications of full strength Phisohex. Treatment with gentian violet appeared to be the more effective.

The water system used for this experiment was exactly the same as described for Experiment 3.

Results and Interpretations

The weights of each hatchling during the experiment is given in Table 9A. This table indicates the difference in size between the turtles of Group 1 and of Groups 2 and 3. Mortality was highest in Group 1 with 2 of the 4 hatchlings dying. Figure 4A shows the rate of growth of each group. Group 1, which received the amino acid diet (Diet 021), lost weight during the first week but gained weight during the remainder of the experiment. As can be seen from Table 9A, much of this later apparent gain is the result of the deaths of the 2 turtles which had lost a large percentage of their initial weight. This left the 2 turtles which had gained weight and therefore raised the

Table 8A. Chemical composition of Diets 021, 022, and 023

Component	gm/100 gm of diet		
	Diet 021	Diet 022	Diet 023
L-Lysine • HCl	1.65		
L-Histidine • HCl • H_2O	.33		
L-Arginine • HCl	1.58		
L-Aspartic Acid	2.06		
L-Threonine	.72		
L-Serine	.74		
L-Glutamic Acid	3.08		
L-Proline	.65		
Glycine	.82		
L-Cystine	.19		
L-Valine	.78		
L-Methionine	.49		
L-Isoleucine	.67		
L-Leucine	1.51		
L-Tyrosine	.72		
L-Phenylalanine	.78		
L-Tryptophan	.17		
L-Alanine	1.06		
Amino Acid Total	18.00		
Vitamin Free Casein		7.2	14.4
Vitamin Diet Fortification Mixture	2.5	2.0	2.0
Dextrose	30.0	24.0	24.0
Hawk Oser Salt Mixture No. 3	1.4	1.1	1.1
Corn Oil	2.0	1.6	1.6
Sodium Bicarbonate*	2.0		
Agar*	3.0	2.4	2.4
Water (Distilled)	41.1	61.7	54.5

*Diet 021 was modified after 2 days by increasing the amount of agar to 4% and lowering the percentage of sodium bicarbonate to 1.0%

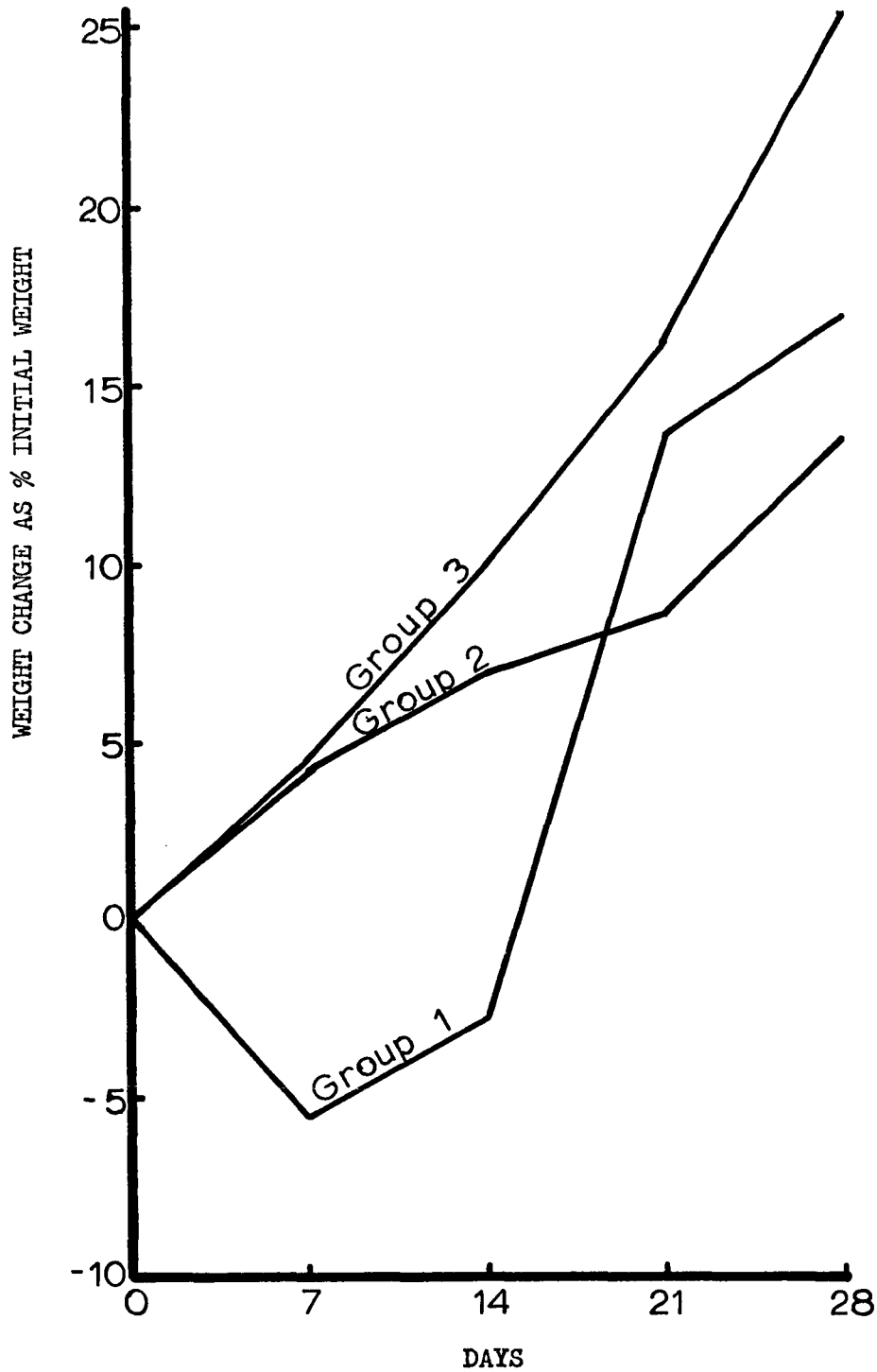


Figure 4A. Average weight change of turtles in each group of Experiment 4 as a percentage of their initial weight

Table 9A. Individual weights in grams of turtles in each group of Experiment 4 during experimental period

Group	Initial	7 days	14 days	21 days	28 days
Group 1					
# 1	52.9	52.7	56.2	59.3	60.4
# 2	57.8	53.8	49.1	dead	dead
# 3	79.1	81.4	79.8	91.1	94.6
# 9	82.4	69.3	dead	dead	dead
Group 2					
#16	221.4	243.1	256.0	256.1	276.3
#20	105.3	106.0	105.6	105.7	108.5
#23	172.5	176.4	181.1	189.4	194.4
#26	78.7	dead	dead	dead	dead
Group 3					
#11	158.6	161.1	165.6	171.4	186.1
#12	121.4	131.0	141.3	152.6	170.5
#13	111.7	126.5	137.6	140.4	149.0
#24	156.9	154.0	159.1	172.8	182.0

percent of initial weight gain value for this group as is shown in Figure 4A. Average food ingestion increased continuously in this group but the low initial values for food consumption in this group are probably a result of the 2 turtles (which eventually died) not eating but having their presence reduce the amount actually ingested by the 2 healthy turtles.

Group 3, fed the diet containing the 14.4% casein level, gained almost twice as much (percent weight increase) as did Group 2, fed the low level (7.2%) casein. The efficiency of Diet 023, calculated by dividing the average weight gain of surviving turtles by the average amount of food consumed per turtle, was 15.6% while the efficiency of Diet 022 was 11.1%. If diet efficiency is calculated by dividing average weight gain by the amount of casein ingested, then efficiency of the low protein diet (Diet 022) becomes greater (154.3%) than that of Diet 023 (the high protein diet) at 108.8%.

In summary, results indicated that increasing the level of dextrose from 20-30% had no effect on growth. The effect of sodium bicarbonate on diet palatability could not be determined from the results. Decreasing the level of casein in the diet by 50% resulted in decreased growth.

Experiment 5

Objectives

This experiment was designed to determine: (1) if unhydrolyzed vitamin-free casein when mixed in various amounts with the amino acid mixture would result in a diet which would be more suitable for

determination of nutritionally essential amino acids for the hatchling sea turtle, (2) the effect of using potato starch as a carbohydrate source in place of dextrose, (3) the effect of lowering the level of carbohydrate and simultaneously raising the level of fat in the form of corn oil, (4) the effect of the addition of sodium bicarbonate to the diet; and (5) to test the effectiveness of 2 different levels of dextrose in the diet.

Materials and Methods

The turtles used in this experiment were Pacific Ridley hatchlings, Lepidochelys olivacea, hatched in the Tucson laboratory from eggs obtained by the author with the assistance of the Instituto Nacional de Pesca from the turtle nesting beach, Playa del Piedra del Tlacoyunque, located near Tecpan, Guerrero, Mexico. Ridley hatchlings were used since it was impossible to obtain Green sea turtle hatchlings at this time. It was assumed that the basic nutritional requirements of all sea turtle hatchlings were similar. The hatchlings were placed in artificial sea water 2 days after hatching and were not fed until the experiment was begun 11 days later (hatchlings which emerged later, of course, had a shorter period before the experiment).

The 66 oldest hatchlings were divided into 11 groups of 6 hatchlings each. The hatchlings were individually weighed and a number was painted on the plastron using a felt-tipped marker. The hatchlings were individually weighed at the beginning of each week of the experiment.

This experiment was conducted in an enclosed laboratory at the University of Arizona campus. The water system consisted of a wooden tank 1.2 m x 2.4 m in size. The tank was constructed of 3/4 inch plywood and was painted with 2 coats of epoxy resin. The water system had a total volume of approximately 950 liters. A solution heating and cooling unit as described in Experiment 1 was utilized to circulate water in the system and to control the temperature. Water flowed from near the bottom of one end of the tank to the unit and was then pumped through the unit and back to the opposite end of the tank where it was released near the surface of the water. The hatchlings were kept in small pens placed inside the large tank. The pens consisted of wooden frames approximately 92 cm x 50 cm x 30 cm covered with plastic mosquito screen and divided into 3 compartments. The wooden frames were painted with epoxy resin. The pens were placed into the large tank in such a way that the water depth was about 20 cm. The large tank had 12 compartments for hatchlings and each had a Biozonic 3-stage power filter attached. These filters consist of a plastic filter chamber containing a foam block unit and an activated charcoal unit. Water flow into the filter was by a siphon while water was pumped out of the filter and into the hatchling compartment by a magnetic-coupled pump at a rate of 380 liters/hour. The total filtration rate of the tank was approximately 4560 liters/hour. The filters were cleaned once each week. Rila brand synthetic sea salt was mixed with tap water to produce a salinity of about 32°/oo. Water temperature was maintained at approximately 26°C.

Group 1 received Diet 024 containing 18% unhydrolyzed vitamin-free casein, Group 2 received Diet 025 with 13.5% casein and 4.5% amino acid mix, Group 3 was fed Diet 026 containing 9% casein and 9% amino acid mixture, Group 4 received Diet 027 with 4.5% casein and 13.5% amino acid mix, and Group 5 was fed Diet 028 with 18% amino acid mix. Group 6 received Diet 029 which was similar to Diet 028 except the amount of dextrose was decreased from 20% to 10%. Group 7 received Diet 030 which was similar to Diet 028 except it contained 1% sodium bicarbonate and 4% agar in place of the normal 3% agar level. Group 8 was fed Diet 031 which was like Diet 030 except the level of dextrose was decreased from 20% to 10%. Group 9 received Diet 032 containing 20% potato starch as the carbohydrate in place of dextrose and with 18% amino acid mixture. Group 10 received Diet 033 with the level of corn oil increased from 2% to 7% and the level of dextrose decreased from 20% to 10%. Group 11 was fed chopped shrimp, Diet 034. The composition of the amino acid mixture used is given in Table 10A. Table 11A gives the composition of Diets 024-033.

The method of feeding the synthetic diets was very different from that used in previous experiments. After the diet was completely prepared, it was poured into a 50 ml. plastic syringe which had been heated to 65°C. A 25 cm. section of 10 mm. glass tubing was then attached using a short piece of plastic hose to the syringe. The still-fluid diet was then forced into the glass tubing (about 20 grams of diet/tube). Tubes of diet were stored in a refrigerator until used. For feeding, the section of glass tubing was supported by a hole in a cork nailed to the edge of the container holding the hatchlings. The

Table 10A. Composition of amino acid mixture used in Diets 025-033

Amino acid	gm/100 gm of amino acid mixture
L-Lysine • HCl	9.1
L-Histidine • HCl • H_2O	1.8
L-Arginine • HCl	8.7
L-Aspartic acid	11.3
L-Threonine	4.0
L-Serine	4.8
L-Glutamic acid	17.0
L-Proline	3.6
Glycine	4.5
L-Cystine	1.1
L-Valine	4.3
L-Methionine	2.7
L-Isoleucine	3.7
L-Leucine	8.3
L-Tyrosine	4.0
L-Phenylalanine	4.3
L-Tryptophan	1.0
L-Alanine	5.8

Table 11A. Chemical composition of Diets 024-033. Composition of amino acid mixture is given by Table 10A.

Ingredient	gm/100 gm of diet									
	Diet 024	Diet 025	Diet 026	Diet 027	Diet 028	Diet 029	Diet 030	Diet 031	Diet 032	Diet 033
Unhydrolyzed casein (vitamin free)	18.0	13.5	9.0	4.5						
Amino acid mixture		4.5	9.0	13.5	18.0	18.0	18.0	18.0	18.0	18.0
Dextrose	20.0	20.0	20.0	20.0	20.0	10.0	20.0	10.0		10.0
Potato starch									10.0	
Vitamin Diet Fortification Mixture	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Hawk Oser Salt Mixture No. 3	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	7.0
Sodium bicarbonate							1.0	1.0		
Agar	3.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	3.0	3.0
Distilled water	53.1	53.1	53.1	53.1	53.1	63.1	51.1	61.1	53.1	58.1

lower end of the glass tube was placed at the surface of the water. A small cork placed in the tube made it possible to push the diet from the tube with a glass rod. As the hatchlings ate the exposed diet, more was pushed beyond the edge of the tube. Turtles were fed 6 days each week, not being fed the day before weighing. It was hoped that this feeding method would reduce the time that the diet was in the water before being eaten, thereby reducing the degree of leaching of nutrients from the ration.

Results and Interpretation

The weight of hatchlings in each group during the 4 weeks of the experiment is given in Table 12A. This table also indicates that only 4 hatchlings died during the course of the experiment. The average rate of growth of hatchlings in each group expressed as percent of initial weight/week is shown in Figure 5A. This figure shows that Group 11, receiving the diet of shrimp, by far had the greatest rate of weight gain. The groups receiving the synthetic diets varied in percent of initial weight gain from 15.4% for Group 5 to 27.4% for Group 9. Food consumption for each group reached its maximum during the third week, after which feeding decreased. The average amount of synthetic diet consumed per turtle during the 4 weeks of the experiment ranged from 5.4 grams for Groups 1 and 10 to 15.8 grams for Group 9. Food conversion efficiency was greatest on Diet 033 with a value of 59.2%. This diet had a greater percent corn oil than did the other diets, which might explain its high conversion, but this group gained

Table 12A. Individual weight in grams of turtles in each group of Experiment 5 during experimental period

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 1					
# 1	15.8	16.9	18.1	20.1	19.4
# 2	17.4	18.1	18.7	19.6	18.7
# 3	16.4	16.7	16.6	16.5	18.3
# 4	16.5	17.3	18.7	18.4	dead
# 5	17.5	17.8	18.7	18.9	18.0
# 6	17.0	17.6	21.1	23.0	23.4
Group 2					
# 7	15.0	16.7	17.3	18.2	17.3
# 8	15.0	15.5	15.9	17.5	18.8
# 9	16.0	16.4	16.8	18.0	17.5
#10	17.0	17.8	19.5	23.1	22.6
#11	13.9	14.4	14.9	14.9	15.9
#12	15.3	16.0	16.2	16.2	dead
Group 3					
#13	16.6	17.5	20.2	19.9	20.7
#14	17.0	18.8	20.7	22.1	23.7
#15	17.2	18.0	19.0	18.6	16.6
#16	17.6	17.2	17.5	17.3	20.0
#17	17.5	17.7	18.1	17.5	19.3
#18	16.7	18.5	20.4	20.7	19.8
Group 4					
#19	18.4	18.9	19.4	19.2	19.0
#20	16.1	16.3	17.2	18.4	19.4
#21	16.8	16.5	17.5	16.7	16.2
#22	15.8	16.6	18.6	20.8	20.7
#23	15.9	16.3	16.8	17.2	17.3
#24	15.8	19.1	20.8	22.0	25.1
Group 5					
#25	15.6	17.0	17.9	18.7	19.0
#26	15.9	16.4	16.9	17.5	18.5
#27	17.3	19.2	19.5	18.8	19.4
#28	15.7	16.4	16.3	16.5	16.9
#29	17.9	18.5	19.5	20.1	19.0
#30	16.4	17.4	18.1	18.3	21.2

Table 12A, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 6					
#31	17.3	18.7	18.0	18.9	19.6
#32	17.8	18.0	18.3	18.6	18.0
#33	16.4	17.0	17.4	17.6	18.1
#34	16.0	16.6	16.9	18.3	19.5
#35	17.1	18.5	18.7	20.2	23.1
#36	18.3	19.0	19.2	19.2	21.2
Group 7					
#37	16.4	18.5	19.8	20.1	21.9
#38	17.3	17.6	18.8	18.6	18.7
#39	14.9	16.0	16.3	17.6	18.1
#40	14.7	15.6	15.9	17.0	17.4
#41	16.8	17.5	18.0	19.0	18.4
#42	13.9	15.8	16.8	17.8	18.3
Group 8					
#43	18.1	19.0	16.1	dead	dead
#44	16.5	18.8	19.9	22.1	23.5
#45	15.3	17.1	17.8	18.8	19.0
#46	14.6	15.6	16.0	16.1	16.2
#47	15.8	16.6	17.1	19.0	16.5
#48	18.5	19.4	21.0	22.8	24.1
Group 9					
#49	17.4	18.4	19.7	20.9	22.2
#50	16.1	18.7	18.9	19.8	21.9
#51	13.8	14.4	dead	dead	dead
#52	15.5	16.8	18.7	19.1	21.3
#53	17.1	18.4	18.7	18.5	17.9
#54	15.9	17.3	18.9	20.3	21.2
Group 10					
#55	16.4	17.6	18.3	20.0	20.1
#56	16.7	17.8	18.5	19.6	21.8
#57	16.1	16.6	17.3	18.5	18.8
#58	18.0	18.4	19.2	19.1	19.6
#59	17.1	17.6	18.1	18.9	20.5
#60	14.3	15.4	16.1	16.1	16.8

Table 12A, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 11					
#61	18.1	20.6	25.3	28.4	33.3
#62	17.4	22.1	26.7	28.6	34.7
#63	16.8	21.4	25.8	28.7	31.8
#64	17.6	21.7	24.0	26.1	29.0
#65	16.6	17.1	18.7	21.4	23.9
#66	17.9	21.4	25.5	28.4	33.0

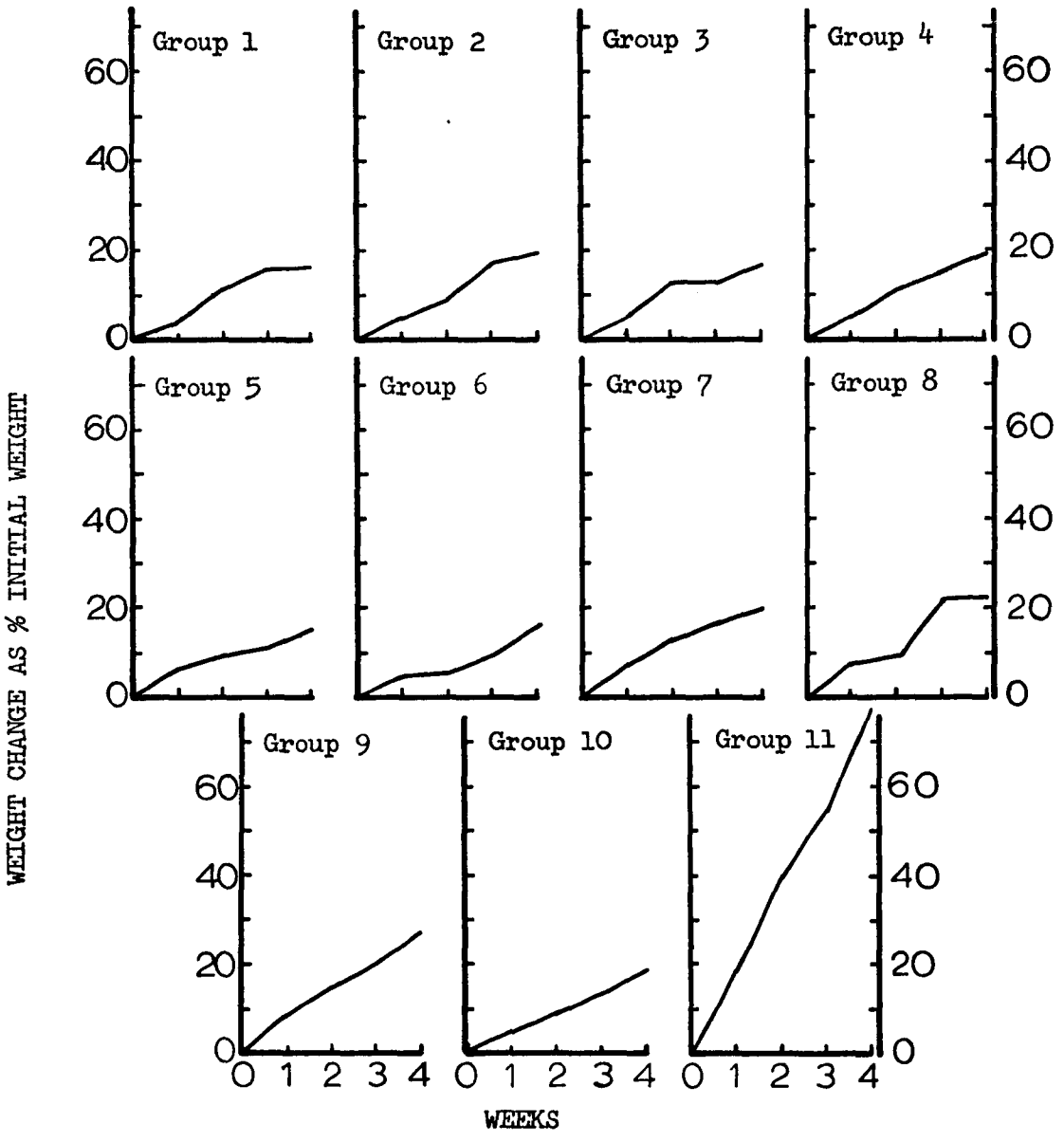


Figure 5A. Average weight change of turtles in each group of Experiment 5 as a percentage of their initial weight

very little and therefore was not successful. The shrimp (Diet 034) had a conversion rate of 53.4%.

The interpretation of the results of this experiment were incorrect in that the percent of initial weight gain value for Group 9 was miscalculated to be 53.2% rather than the correct value of 27.4%. Using this erroneous data, it was concluded that potato starch was far superior to dextrose as a carbohydrate source when in fact this diet resulted in only a 12% gain over that obtained using dextrose. On the basis of this, it was decided that starch was preferable to dextrose for the carbohydrate component of the test diets. This mistake in calculation was not discovered until a late date and therefore all diets after Experiment 5 utilized potato starch as the carbohydrate source. While this comprises a meaningless complication, it has no apparent bearing on the central issue of determination of essential amino acids; in fact, the lower leaching rate of potato starch presumably improves the synthetic diet.

In summary, results indicated that mixing casein and amino acid mixture in various proportions did not improve growth. The effect of lowering carbohydrate level from 20-10%, of substituting corn oil for a portion of carbohydrate level, and of sodium bicarbonate on diet palatability could not be determined from experimental results.

Experiment 6

Objective

This experiment was designed to test the effect of protein level on palatability of synthetic diets and to measure resulting weight change.

Materials and Methods

The turtles used were survivors of Experiment 5. After the termination of the prior experiment, the L. olivacea hatchlings were transported to the facilities at Puerto Penasco, Sonora, Mexico. They were fed on chopped shrimp for 31 days, then returned to the Tucson laboratory. The 15 hatchlings which appeared to be in the best physical condition were divided into 3 groups of 5 hatchlings each. The turtles were individually weighed and a number was placed on the plastron.

Group 1 received Diet 035 (identical to Diet 032, Experiment 5) which contained 18% amino acid mixture (Table 10A) and 20% potato starch. Group 2 received Diet 036 which differed from Diet 035 in that it contained 36% instead of 18% amino acid mixture. Group 3 was fed Diet 037, chopped shrimp. Diet preparation, feeding procedure, and water system was as described for Experiment 5.

Results and Interpretation

Group 1 ingested a total of 69.3 grams of Diet 035, Group 2 ingested 121.5 grams of Diet 036, and Group 3 ingested 40.3 grams of shrimp during the first week of the experiment (6 feeding days). As this indicates, Diet 036 with the 36% protein level was ingested at almost twice the rate of Diet 035 with 18% protein. Palatability therefore seems to be directly proportional to level of protein in the synthetic diet.

The surprising aspect of this experiment was that Group 1 lost weight from an average of 38.9 grams to 37.5 grams while Group 2, even after having ingested such large quantities of the high protein diet,

gained little with an increase in average weight from 34.0 to 34.6 grams. The failure of these groups to do well could not be explained. The experiment was terminated, since it was obvious that some factor in the synthetic diets was still not suitable. It was found that the salt mixture contained less sodium, potassium, phosphorous, and chlorine than did shrimp flesh, and therefore it was decided to add 0.4% potassium phosphate monobasic and 0.2% sodium chloride to future synthetic diets.

Experiment 7

Objective

The objective of this experiment was to determine if the amino acids glycine, L-proline, L-arginine, L-isoleucine, L-histidine, and L-threonine are nutritionally essential or non-essential for the hatchling Green sea turtle.

Materials and Methods

The Green sea turtle hatchlings used in this experiment were obtained from Dr. Robert Bustard, Australian National University. The hatchlings were maintained in the Tucson laboratory for 15 days prior to beginning the experiment and during this time were fed chopped shrimp. The 40 hatchlings in the best physical condition were divided into 8 groups of 5 hatchlings each. The hatchlings were individually weighed and tagged at the base of the right postcentral lamina with a No. 1 size monel metal tag (National Band and Tag Co.). The turtles of each group were weighed at the beginning of each week of the experiment.

The synthetic diets contained 18% protein supplied by an amino acid mixture, 20.0% potato starch, 2.5% Vitamin Diet Fortification mixture, 2% corn oil, 1.4% Hawk Oser Salt Mixture No. 3, 0.4% potassium phosphate monobasic, 0.2% sodium chloride, 3.0% agar, and 52.5% distilled water. The amino acid mixture used for the control group (Group 1) was as given in Table 10A. The amino acid mixtures used in each of the deleted diets differed in that the test amino acid was simply not added to the other 17 amino acids. No filler substance was used to replace the deleted amino acid; therefore non-deleted amino acids occurred in a slightly higher individual concentration than in the control diet.

Group 1 received the control diet, Diet 038. Group 2 was fed Diet 039, the L-proline-free diet. Group 3 was fed Diet 040, the L-histidine-free diet. Group 4 received Diet 041, a L-threonine-free diet. Group 5 received the glycine-free diet, Diet 042. Group 6 was fed the L-isoleucine-free diet, Diet 043. Group 7 received Diet 044, a L-arginine-free diet. Group 8 was fed chopped shrimp, Diet 045. Diet preparation and feeding procedure was the same as in Experiments 5 and 6, with the exception that the hatchlings were fed 7 days a week.

The water system was the same as for Experiments 5 and 6. Malachite green was added to the water at irregular intervals to maintain water clarity. The amount used was 40.0 ml of 0.5% malachite green solution.

Results and Interpretation

The individual weights of the hatchlings during the 2 weeks of the experiment are given in Table 13A. The average percent of initial

Table 13A. Individual weights in grams of turtles in each group of Experiment 7 during experimental period

Group	Initial	1 Week	2 Week
Group 1			
#104	31.3	33.6	33.1
#127	29.7	30.8	31.6
#133	30.9	32.9	35.5
#140	28.3	31.1	33.9
#143	28.3	31.0	31.7
Group 2			
#101	32.3	33.5	34.4
#103	28.3	29.9	29.3
#111	32.5	36.9	39.2
#124	32.7	33.2	34.3
#125	31.8	37.8	39.2
Group 3			
#107	28.3	30.0	25.0
#108	33.0	34.7	33.4
#114	30.6	32.2	31.5
#119	31.1	32.8	32.1
#149	30.0	29.7	dead
Group 4			
#115	31.9	33.9	34.8
#118	32.8	32.0	31.0
#121	29.9	29.3	28.4
#126	35.5	36.1	34.9
#142	28.8	27.3	22.8
Group 5			
#105	30.9	31.5	30.4
#110	32.0	32.2	33.5
#130	27.6	30.6	27.9
#135	31.4	31.9	30.6
#137	32.0	32.2	32.4
Group 6			
#106	34.8	35.1	35.0
#117	31.4	31.1	31.5
#120	31.9	30.4	28.7
#136	33.4	33.6	36.9
#138	31.9	30.7	28.0

Table 13A, Continued

Group	Initial	1 Week	2 Week
Group 7			
#123	31.8	32.9	33.6
#129	32.7	33.7	32.7
#131	31.4	32.9	29.4
#141	30.3	31.7	31.8
#146	30.7	31.2	31.0
Group 8			
#102	33.1	39.8	46.3
#113	26.3	34.4	41.8
#122	30.7	40.7	47.0
#144	34.9	40.4	50.9
#147	30.3	33.9	38.2

weight change for each group is given in Table 14A. From Table 13A it can be seen that the initial group weights were similar and that only 1 hatchling died. Table 14A indicates that during the first week all groups except those receiving the L-threonine-free diet or the L-isoleucine-free diet gained weight, with the greatest weight gain being on the shrimp diet and on the control and L-proline-free synthetic diets. By the end of the second week only the groups receiving the control or L-proline-free diets or the shrimp diet had continued to gain weight, although the rate of gain for the two synthetic diet groups was less than during the first week. It would appear that the main cause for the failure of the synthetic diet groups to do well, realizing of course that at least a few of the tested amino acids should be nutritionally essential, was that food consumption dropped from an average intake of 53.3 grams/group/week during the first week to an average of only 12.4 grams/group/week during the second week. This decrease in feeding was observed for Groups 1 and 2 as well as the other synthetic diet groups. No valid conclusions could be reached from this data concerning the essential or non-essential nature of the tested amino acids; however, the results at least suggest that L-proline may be non-essential while L-threonine and L-isoleucine appear to be essential amino acids.

Experiment 8

Objective

The purpose of this experiment was to determine the effectiveness of 4 different amino acid mixtures when utilized as the protein source in synthetic diets for hatchling sea turtles.

Table 14A. Average percent of initial weight change for each group of Experiment 7

Group	Week 1	Week 2
Group 1	7.3%	11.6%
Group 2	8.7%	11.9%
Group 3	4.2%	-0.8%
Group 4	-0.2%	-5.0%
Group 5	2.9%	0.6%
Group 6	-1.6%	-2.0%
Group 7	3.5%	1.0%
Group 8	21.2%	44.4%

Materials and Methods

The turtles used were loggerhead hatchlings (Caretta caretta gigas) from South Africa. The hatchlings arrived in Tucson 15 days before the experiment was begun and were fed shrimp during this period. The turtles were individually weighed and tagged as described for Experiment 7. The experiment consisted of 4 groups of 5 hatchlings each, which were to receive the synthetic diet, and 1 group containing 2 hatchlings which were to receive chopped shrimp. Only 2 turtles were used in the shrimp fed group, since the feeding of large quantities of shrimp made it very difficult to maintain water quality. The turtles were weighed at the beginning of each week.

The synthetic diets contained different amino acid mixtures. Since the qualitative amino acid requirements had been determined for several different animal species, it was thought that perhaps the amino acid mixtures utilized by these investigators would be suitable for hatchling sea turtles. Group 1 received Diet 046, a diet containing 18% of an amino acid mixture used in chicken amino acid studies (Allen et al., 1972). Group 2 was fed Diet 047 containing 18% of an amino acid mixture developed by Halver (1957) for use with chinook salmon. Group 3 received Diet 048. This diet contained a "squid" modification of the amino acid mixture given in Table 10A. In comparing the amino acid pattern given in Table 10A to the amino acid pattern of squid (as determined by the author), it was noted that squid, which in experiments conducted both in Puerto Penasco, Mexico and Tucson seemed to be superior to shrimp in terms of growth-promoting properties, contained a greater concentration of both glycine and L-threonine. For

this reason, the amino acid mixture given in Table 10A was modified by adding additional L-threonine and glycine. Group 4 received Diet 049 containing 18% of the amino acid mixture given in Table 10A. Group 5 received chopped shrimp, Diet 050. The actual composition of the synthetic diets is given in Table 15A.

The diets were again fed (7 days a week) using the glass tubes as already described. Diet preparation was the same as in Experiment 5. The water system was also the same as for Experiment 5. Water temperature fell to 22°C. on one occasion, but remained at approximately 25°C. for most of the experiment. Salinity varied slightly from an initial salinity of 28°/oo.

Results and Interpretation

The weights of the hatchlings during the experiment are given in Table 16A. It can be noted that no hatchlings died during the 2 weeks the experiment was in progress. Table 17A gives the average weight change as percent of initial weight values for each group during the experiment. Turtles in each group gained weight during the first week, with Group 5 on the shrimp diet having greatest growth. During the second week each of the 4 groups receiving the synthetic diets lost weight with Group 1 showing the greatest decrease in weight. The shrimp-fed group continued to gain weight. The cause of the weight loss during the second week seems to be a drop in feed consumption by those groups on the synthetic diets. During the first week the average total consumption (7 days) per group was 84.8 grams. This value decreased during the second week to 48.6 grams/group. The results of this

Table 15A. Chemical composition of Diets 046-049

Ingredient	g/m 100 gm of diet			
	Diet 046	Diet 047	Diet 048	Diet 049
L-Lysine · HCl	.91	1.29	1.50	1.63
L-Histidine · HCl · H_2O	.39	.65	.30	.33
L-Arginine · HCl	.82	1.29	1.43	1.56
L-Aspartic acid	----	1.29	1.86	2.04
L-Threonine	.62	.65	1.76	.71
L-Serine	----	.77	.79	.86
L-Glutamic acid	9.67	2.00	2.76	3.10
L-Proline	.19	1.29	.59	.64
Glycine	1.15	1.29	1.23	.82
L-Crystine	.34	.13	.18	.19
L-Valine	.79	1.03	.70	.77
L-Methionine	.34	.52	.45	.49
L-Isoleucine	.58	1.03	.61	.66
L-Leucine	1.15	1.54	1.37	1.50
L-Tyrosine	.43	1.03	.65	.71
L-Phenylalanine	.48	1.03	.70	.77
L-Tryptophan	.14	.26	.16	.17
L-Alanine	----	.91	.96	1.05
Amino acid subtotal	18.00	18.00	18.00	18.00
Potato starch	20.00	20.00	20.00	20.00
Vitamin Diet Fortification Mixture	2.50	2.50	2.50	2.50
Corn oil	2.00	2.00	2.00	2.00
Hawk Oser Salt Mix No. 3	1.40	1.40	1.40	1.40
Potassium phosphate monobasic	0.40	0.40	0.40	0.40
Sodium chloride	0.20	0.20	0.20	0.20
Agar	3.00	3.00	3.00	3.00
Distilled water	52.50	52.50	52.50	52.50

Table 16A. Individual weights in grams of turtles in each group of Experiment 8 during experimental period

Group	Initial	Week 1	Week 2
Group 1			
#175	28.7	30.0	27.4
#190	28.0	29.0	26.0
#191	26.1	30.6	27.4
#200	28.7	31.5	26.4
#222	26.3	29.1	24.4
Group 2			
#184	27.6	29.7	29.0
#193	28.9	33.7	33.3
#205	28.4	36.1	35.9
#220	28.5	31.8	31.1
#249	29.3	31.8	29.0
Group 3			
#179	27.6	29.1	29.6
#204	25.1	25.8	25.3
#207	28.7	34.0	31.6
#219	26.5	30.8	30.0
#230	25.9	30.5	27.8
Group 4			
#203	29.1	33.7	33.9
#206	28.8	32.2	31.9
#228	28.2	29.7	25.6
#229	27.9	28.5	28.6
#265	27.2	32.7	32.4
Group 5			
#180	27.9	33.7	39.5
#189	25.8	29.4	34.0

Table 17A. Average percent of initial weight change for each group of Experiment 8

Group	Week 1	Week 2
Group 1	9.0%	-4.5%
Group 2	14.3%	10.9%
Group 3	12.3%	7.8%
Group 4	11.1%	7.9%
Group 5	17.5%	36.9%

experiment indicated that some factor was still making the synthetic diets unsuitable. Since Diets 047, 048, and 049 gave similar results and since all were apparently better than Diet 046, it was decided to continue using the amino acid mixture given in Table 10A.

Since the turtles on synthetic diets had stopped feeding for all practical purposes, the experiment was terminated at the end of the second week. One turtle each from Groups 1, 2, 3, and 4 was dissected and examined by Dr. Sheldon, a faculty member of the Animal Pathology Department at The University of Arizona. Dr. Sheldon reported that the turtles fed the synthetic diets (one from each group) had the lower intestinal tract blocked with what appeared to be agar.

Experiment 9

Objectives

The main objective of this experiment was to test the suitability of three different binding agents. The agents tested were agar, carboxymethyl cellulose sodium, and gelatin. A secondary objective of this experiment was to determine the relative effectiveness of a hydrolyzed protein source (hydrolyzed casein) to an unhydrolyzed protein source (vitamin-free casein) when used in prepared diets for hatchling sea turtles.

Materials and Methods

The turtles used in this experiment were C. mydas hatchlings from eggs laid on the turtle-nesting beaches of Surinam. The eggs were collected with the cooperation of Dr. J. Schulz, Forest Department,

Surinam and transported to the facilities of Mariculture, Ltd., a sea turtle farm located on Grand Cayman Island, British West Indies. The eggs were hatched at Mariculture, Ltd. and the hatchlings were sent by air to Tucson. The hatchlings were fed chopped shrimp during the 15 day period before the experiment was begun. All the hatchlings appeared to be in excellent physical condition. The turtles were divided into 6 groups of 5 hatchlings each and individually weighed and tagged as previously described in Experiment 7. The hatchlings of each group were weighed at the beginning of each week of the experiment.

The water system remained basically the same as described for Experiment 5, with the exception that new compartments were made to fit into the large tank. These compartments were the same size as those used in Experiment 5, but were made with solid wooden sides and partitions with a plastic screen bottom. This prevented turtles in one group from seeing what turtles in an adjacent group were being fed. In the past, turtles next to the shrimp-fed groups would sometimes ignore the food in their compartment and try to reach the uneaten shrimp in the next compartment. The water return from the heating and cooling unit to the large tank was also somewhat different in that, instead of just returning the water to the end opposite the out flow, the water was sprayed into the line of compartments by holes drilled along the length of a plastic pipe running down the center of the large tank. This resulted in a continuous flow of water through the bottom of the enclosed compartments. The water was changed once during the experiment, at the end of the first 2 weeks. The filters were cleaned each week. As

described in previous experiments, 40 mls of 0.5% malachite green solution was added twice during the experiment to reduce the number of bacteria and improve water clarity.

Group 1 received Diet 051, a 18% amino acid mixture diet bound with agar. Group 2 received Diet 052, a 18% amino acid mixture diet bound with carboxymethyl cellulose sodium. Group 3 received Diet 053 containing 18% protein (2% gelatin and 16% amino acid mixture) and was bound by the contained gelatin. The composition of the 16% amino acid mixture was such that when it was added to 2% gelatin the combined amino acid pattern would be the same as that used in Diets 051 and 052. Group 4 received Diet 054 made with 18% unhydrolyzed casein bound with carboxymethyl cellulose sodium. Group 5 was fed Diet 055, 18% hydrolyzed casein bound with carboxymethyl cellulose. Group 6 received chopped shrimp, Diet 056. The composition of the synthetic diets is given in Table 18A.

The agar diet was prepared by heating the water and agar to 85-90°C. The mixture was allowed to cool to approximately 65°C. before the remaining ingredients, including the vitamin mix, were added. The carboxymethyl cellulose (CMC) diets were made by mixing all the dry ingredients except the CMC. Water heated to 90°C. was then added and mixed. The CMC was added next and thoroughly mixed. The gelatin diet was prepared by heating all ingredients except the vitamin mixture to 85°C. and then, after removing from the heat, adding the vitamin mixture and blending the diet thoroughly. All diets, after being completely mixed and before they could become cooled, were injected into glass tubes as previously described. After 5 days, the level of

Table 18A. Chemical composition of Diets 051-055

Ingredient	gm/100 gm of diet					
	Diet 051	Diet 052	Diet Gelatin*	AA mix	Diet 054	Diet 055
L-Lysine • HCl	1.63	1.63	.09	1.54		
L-Histidine • HCl • H_2O	.33	.33	.02	.31		
L-Arginine • HCl	1.56	1.56	.15	1.41		
L-Aspartic acid	2.04	2.04	.13	1.91		
L-Threonine	.71	.71	.03	.68		
L-Serine	.86	.86	.08	.78		
L-Glutamic acid	3.10	3.10	.25	2.85		
L-Proline	.64	.64	.35	.29		
Glycine	.82	.82	.45	.37		
L-Cystine	.19	.19	trace	.19		
L-Valine	.77	.77	.06	.71		
L-Methionine	.49	.49	.01	.48		
L-Isoleucine	.66	.66	.04	.62		
L-Leucine	1.50	1.50	.07	1.43		
L-Tyrosine	.71	.71	trace	.71		
L-Phenylalanine	.77	.77	.04	.73		
L-Tryptophan	.17	.17	.01	.16		0.2
L-Alanine	1.05	1.05	.22	.83		
Amino acid total	18.00	18.00	2.00	16.00		0.2
Vitamin free casein					18.00	
Hydrolyzed casein						17.80
Potato starch	20.00	20.00	20.00		20.00	20.00
Vitamin Diet Fortification Mixture	2.50	2.50	2.50		2.50	2.50
Hawk Oser Salt Mix No. 3	1.40	1.40	1.40		1.40	1.40
Potassium phosphate monobasic	.40	.40	.40		.40	.40
Sodium chloride	.20	.20	.20		.20	.20
Corn oil	2.00	2.00	2.00		2.00	2.00
Agar	3.00					
Carboxymethyl cellulose sodium		5.00			5.00	5.00
Distilled water	52.50	50.00	55.50		50.50	50.50

*Most of the values given for gelatin were obtained from a University of Arizona food composition table. Values for proline, alanine, serine, aspartic acid, and glutamic acid from Idson and Braswell, 1957. The value for tryptophan is an estimation.

carboxymethyl cellulose in Diets 054 and 055 was reduced from 5% to 3% in order to make it easier to inject the diets into the tubes. The turtles were fed 7 days each week, several (4-8) times each day. Diets were stored in a refrigerator.

Results and Interpretations

The experiment lasted for a 6 week period. The weights of the hatchlings during this period are given in Table 19A. This table indicates that the groups were initially similar in total weight. Hatchling #304 of Group 2 died during the first day of the experiment and therefore can be considered as never having actually been in the experiment. Only one other turtle died during the experiment and that was turtle #276 of Group 1, which died during the last week of the experiment. The average weight change as percent of initial weight for each group is given for the experimental period in Figure 6A. The shrimp-fed group (Group 6) had the greatest rate of growth followed closely by Group 4, fed the unhydrolyzed casein diet (Diet 054). During the last 2 weeks the rate of weight increase declined for Group 6. It is possible that the vitamin level of pure shrimp flesh becomes limiting as the hatchling becomes larger and has utilized all yolk stores. During the last 2 weeks, Group 4 began to approach the level of growth observed on the shrimp diet and had the experiment continued for a few more weeks, the growth on Diet 054 could have surpassed that on shrimp. Figure 6A shows that the hydrolyzed casein diet (Diet 055) did not promote growth to the extent that Diet 054 did, suggesting that unhydrolyzed protein source is better than the same protein after having been

Table 19A. Individual weights in grams of turtles in each group of Experiment 9 during experimental period

Group	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group 1							
#272	36.5	42.8	45.8	50.2	51.9	53.8	51.9
#276	31.7	37.0	40.2	43.2	47.2	42.5	dead
#286	31.2	36.7	40.2	43.4	44.7	46.4	49.4
#287	33.9	39.0	43.4	46.6	42.8	47.4	50.2
#288	32.3	36.9	38.7	39.1	39.5	39.4	41.1
Group 2							
#283	37.4	46.8	50.8	59.1	64.3	67.3	73.1
#284	39.7	48.3	52.5	58.4	65.7	73.0	76.9
#289	32.8	40.3	46.3	53.3	51.6	56.8	57.3
#304	29.4	dead	dead	dead	dead	dead	dead
#306	33.7	38.8	42.5	48.3	54.5	57.7	63.8
Group 3							
#275	34.4	39.9	41.8	43.4	44.3	47.1	48.2
#277	35.2	39.0	41.5	41.9	43.5	42.4	43.1
#280	34.5	38.6	40.0	42.7	45.0	47.4	47.9
#290	33.2	37.5	41.4	44.7	45.8	50.3	47.4
#292	32.0	35.9	36.0	38.3	41.4	40.7	40.5
Group 4							
#291	30.7	39.5	48.3	59.4	70.7	73.3	84.5
#300	35.2	47.6	58.2	69.2	80.2	92.6	97.0
#301	33.4	42.6	47.4	47.6	57.9	69.6	80.8
#302	33.4	40.3	45.9	55.1	64.8	77.2	87.4
#303	35.1	47.9	58.0	66.5	81.2	100.6	113.1
Group 5							
#293	34.4	43.5	48.0	54.6	60.5	65.2	70.0
#294	30.6	38.9	49.0	53.8	60.6	73.7	81.9
#296	32.6	42.0	51.4	60.4	68.1	79.4	90.3
#298	34.0	40.8	48.4	52.8	62.1	68.4	71.2
#299	33.2	43.4	49.9	56.6	62.2	67.1	74.4
Group 6							
#270	30.9	40.8	48.0	63.0	68.7	75.9	80.6
#271	32.7	44.3	56.1	71.7	89.1	102.7	112.5
#274	33.4	44.1	55.8	73.5	89.5	97.1	104.5
#279	39.8	55.5	62.3	74.5	81.3	81.2	88.9
#282	32.4	44.9	54.7	70.7	81.3	90.0	101.5

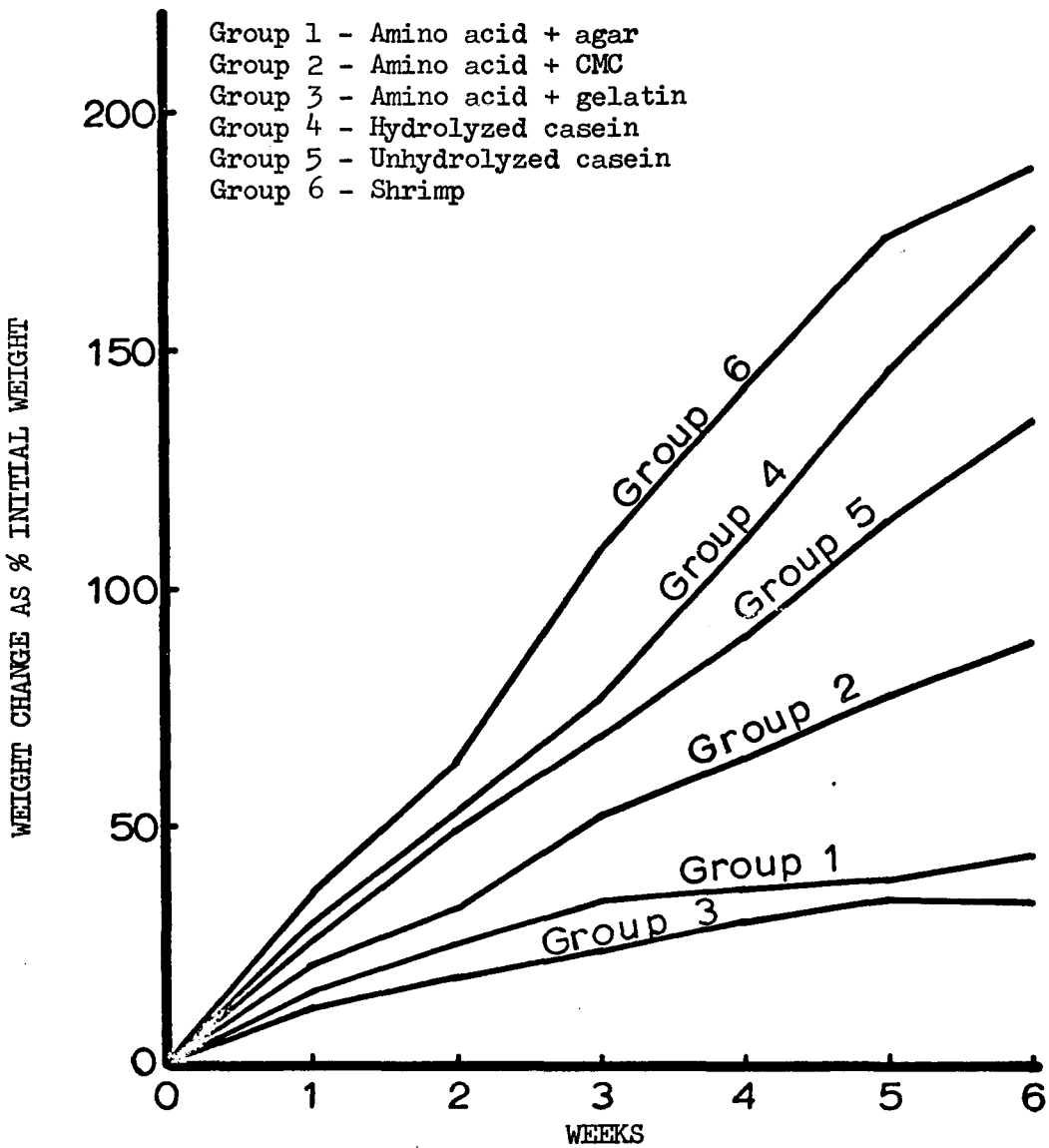


Figure 6A. Average weight change of turtles in each group of Experiment 9 as a percentage of their initial weight

hydrolyzed. Growth results of Groups 1, 2, and 3 show that the diet bound with carboxymethyl cellulose gave approximately twice the growth as that given by diets bound with agar or gelatin. The one turtle that died in the agar-bound group had its gut packed and completely blocked with undigested agar.

If food conversion efficiency is calculated for Diets 051, 052, and 053, it can be seen that efficiency of each of the diets is similar, ranging from 20.7% for Diet 053 to 27.4% for Diet 052. The difference in growth response by Groups 1, 2, and 3 therefore seems to be related to the amount of diet ingested. The agar-bound diet apparently accumulates to a certain extent in the gut and results in a low level of feed intake. The gelatin-bound diet was ingested in approximately the same quantities as the agar-bound diet, but in this case the low level of feeding seems due to palatability; that is, the hatchlings just do not seem to be fond of the diet. The feces of the agar-fed group tended to be relatively hard, while those of the gelatin-bound diet group had a much softer consistency. The unhydrolyzed casein diet was the most efficient in terms of weight gain/gram of diet consumed, with a conversion rate of 66.4%. This compares to 40.5% for Diet 055 and 35% for chopped shrimp.

It was concluded that the use of carboxymethyl cellulose as a binding agent resulted in a diet suitable for the determination of the nutritionally essential amino acids required by the hatchling Green sea turtle. Since the hydrolyzed casein diet (protein component consisting of free amino acids in the pattern as they occur in casein, plus

tryptophan to replace tryptophan destroyed by acid hydrolysis of the whole protein) gave better results than did Diet 052 (protein component consisting of free amino acids in approximately the pattern found in shrimp flesh), it was decided to use the amino acid pattern of casein in future test diets.

Experiment 10

Objective

The objective of this experiment was to test 12 different amino acids to determine if they were nutritionally essential.

Materials and Methods

Green sea turtle hatchlings obtained from Sandakan, Malaysia through the cooperation of Mr. G. S. de Silva, Forest Department, Sandakan were used. The hatchlings arrived in what appeared to be good health, but with some turtles having small patches of a yellowish skin infection. The turtles were fed chopped shrimp with powdered Pervinal vitamin mixture added during the 26 days before the experiment was begun. The hatchlings were divided into 15 groups of 5 hatchlings each. As in previous experiments, the hatchlings were individually weighed and tagged (monel metal tags) at the beginning of the experiment. The turtles were weighed at the beginning of each week of the experiment.

The large number of experimental groups in this experiment made it necessary to modify the water system. The large tank with the enclosed 12 compartments was used as described in the preceding experiment. To provide space for the additional 3 groups, a wooden tank, the size of

one of the pens used to form 3 compartments in the large tank, was divided into 3 parts using opaque white plastic dividers. The resulting 3 containers were the same size as pens in the large tank, thereby giving all groups equal space. Water circulation in this small box was provided by 3 Biozonic filters as described in previous experiments. Groups 13, 14, and 15 were maintained in this small tank.

The diets used in this experiment were basically the same with the exception of amino acid mixture composition. Group 1 received Diet 057 having an amino acid pattern based on the composition of shrimp flesh. This diet is identical to Diet 052 in Experiment 9 and its composition is given in Table 18A. Group 2 received Diet 058 having an amino acid pattern based upon the composition of casein. The percent composition values for the amino acids of casein were based upon an amino acid analysis performed by the author. The actual amino acid pattern was modified to provide approximately equal amounts of lysine and arginine, less cystine, and glutamic acid, and more glycine than in casein. The remaining groups received diets based upon Diet 058. In each case, the amino acid mixture used in Diet 058 was deleted of one amino acid and the amount of nitrogen removed by deletion of the amino acid was replaced by adding diammonium citrate. The groups received the following diets: Group 3, L-lysine-free Diet 059; Group 4, L-histidine-free Diet 060; Group 5, L-arginine-free Diet 061; Group 6, L-aspartic acid-free Diet 062; Group 7, L-threonine-free Diet 063; Group 8, L-serine-free Diet 064; Group 9, L-glutamic acid-free Diet 065; Group 10, L-proline-free Diet 066; Group 11, glycine-free Diet 067;

Group 12, L-alanine-free Diet 068; Group 13, L-cystine-free Diet 069; and Group 14, L-valine-free Diet 070. Group 15 received Diet 071, chopped shrimp. The composition of Diet 058 and the amount of diammonium citrate used to replace deleted amino acid nitrogen in Diets 059-070 (and Diets 073-083 of Experiment 11) is given in Table 20A. Diet preparation and feeding (7 days a week) were as in the last experiment.

Results and Interpretation

During the first week of this experiment 2 hatchlings died. This was followed by 9 more deaths during the second week and 22 additional deaths during the third week. The high mortality (44%) strongly indicated a disease problem since the deaths were almost evenly distributed among the groups, including the shrimp-fed group. Since the number of hatchlings in each group was declining and since the effects of disease could not be distinguished between those of deficient diets, the experiment was terminated at the end of the third week.

Experiment 11

Objective

The objective of this experiment was to determine if the 10 amino acids tested were nutritionally essential for the hatchling Green sea turtle.

Materials and Methods

The hatchlings used were from eggs collected on Ascension Island and hatched at the facilities of Mariculture, Ltd., Grand Cayman Island,

Table 20A. Chemical composition of Diet 058

Ingredient	gm/100 gm of diet	gms of diammonium citrate to replace amino acid nitrogen
L-Lysine · HCl	.72	.89
L-Histidine · HCl · H_2O	.52	.84
L-Arginine · HCl	.67	1.44
L-Aspartic acid	1.32	1.12
L-Threonine	.73	.69
L-Serine	.99	1.06
L-Glutamic acid	4.14	3.17
L-Proline	1.85	1.82
Glycine	.64	.97
L-Alanine	.52	.66
L-Crystine	.14	.13
L-Valine	1.01	.98
L-Methionine	.35	.26
L-Isoleucine	.76	.65
L-Leucine	1.63	
L-Tyrosine	.98	
L-Phenylalanine	.85	
L-Tryptophan	.18	.18
Amino acid subtotal	18.00	
Potato starch	20.00	
Vitamin Diet Fortification Mixture	2.50	
Hawk Oser Salt Mix No. 3	1.40	
Potassium phosphate monobasic	.40	
Sodium chloride	.20	
Corn oil	2.00	
Carboxymethyl cellulose sodium	5.00	
Distilled water	50.50	

British West Indies. The hatchlings arrived in Tucson 20 days before the experiment was begun and were maintained on shrimp during this period. The turtles were transported to the marine laboratory of the Monterrey Institute of Technology located at Guaymas, Sonora, Mexico. The marine lab of the Escuela de Ciencias Maritimas y Technologia de Alimentos in Guaymas offered one major advantage over working in Tucson, that is, large quantities of natural sea water were available and therefore the hatchlings could be maintained in a clean water environment. The turtles were divided into 12 groups of 8 hatchlings each and a shrimp-fed group with only 3 hatchlings. The turtles were individually weighed and tagged at the beginning of the experiment and were re-weighed at the beginning of each week of the experiment.

The water system for this experiment consisted of 13 plastic dish pans, one for each group. These containers held 15 liters of water and were provided with an approximately 1.2 liter/minute flow of sea water. A hole drilled in the bottom of the dish pan held a cork fitted with a piece of glass tubing which served as a drain pipe and maintained a water level of approximately 13 cm. The sea water for this experiment was not filtered and arrived at the tubs at approximately ambient sea water temperature, ranging between 26°C. and 28°C. The water in the system remained very clear; however, it was necessary to clean the tubs twice each week by rinsing with sea water to remove accumulated, semi-dissolved feces from the bottoms of the tubs.

Most of the synthetic diets fed during the first 3 weeks of the experiment were identical to those used in Experiment 10 and given in Tables 18A and 20A. Group 1 received a control diet based on shrimp

amino acid pattern (Diet 072) which was the same as Diets 052 and 057. Group 2 received Diet 073 (casein amino acid pattern), identical to Diet 058. Group 3 was fed Diet 074 (Diet 059), a L-lysine-free diet. Group 4 received Diet 075 (Diet 060), a L-histidine-free diet. Group 5 received Diet 076 (Diet 064), a L-serine-free diet. Group 6 was fed a L-proline-free diet, Diet 077 (Diet 066). Group 7 was fed a glycine-free diet, Diet 078 (Diet 067). Group 8 received Diet 079 (Diet 068), a L-alanine-free diet. Group 9 received Diet 080 (Diet 070), a L-valine-free diet. Group 10 was fed a L-methionine-free diet, Diet 081. Group 11 received a L-isoleucine-free diet, Diet 082. Group 12 was fed a L-tryptophan-free diet, Diet 083. Diets 081, 082, and 083 were not fed in the previous experiment. As with the other deleted diets, the amount of nitrogen removed by deleting the amino acid was replaced using diammonium citrate. The amount of diammonium citrate used in these diets is included in Table 20A. Group 13 was fed chopped shrimp, Diet 084. Diet preparation was the same as in the last experiment. Feeding method was the same as before, using the diet-filled tubes but the turtles were fed for only 6 days a week, not being fed the day prior to weighing.

Results and Interpretation

The weights of the hatchlings during the experimental period are given in Table 21A. The average weight change as percent initial weight is given for each group in Figure 7A. This figure shows that at the end of the third week all the synthetic diet groups with the exception of Group 1 (shrimp patterned amino acid mixture) and Group 8

Table 21A. Individual weights in grams of turtles in each group of Experiment 11 during experimental period

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 1					
#384	37.4	43.8	45.6	47.8	50.7
#385	34.8	38.9	41.6	43.9	45.4
#386	34.1	38.6	40.6	43.5	46.1
#387	28.4	32.7	37.0	40.0	41.0
#388	40.5	44.5	47.7	52.9	54.7
#389	38.8	44.7	45.0	51.7	55.4
#390	42.1	46.5	52.3	55.0	55.8
#391	33.8	38.0	41.8	47.3	51.2
Group 2					
#392	42.1	47.7	44.7	42.0	49.2
#393	33.4	36.1	35.7	35.7	37.9
#394	34.4	36.2	35.3	34.9	37.6
#395	35.6	38.8	36.2	35.4	39.8
#396	45.1	48.3	47.3	46.8	dead
#397	36.5	39.6	39.6	39.0	46.5
#398	36.9	39.9	39.0	38.8	41.0
#399	36.0	38.6	36.8	35.0	dead
Group 3					
#400	36.1	38.0	36.5	dead	dead
#401	34.8	38.1	36.7	35.4	36.3
#402	32.8	36.0	35.0	34.8	31.7
#403	36.5	39.6	40.4	40.8	39.2
#404	36.8	39.8	37.3	36.7	dead
#405	38.4	40.6	39.3	39.6	dead
#406	36.6	41.3	39.3	39.2	39.2
#407	31.8	33.9	32.9	32.4	32.0
Group 4					
#408	34.0	37.5	38.0	38.8	40.4
#409	36.7	38.8	37.0	37.1	38.5
#410	38.3	39.8	39.1	39.4	dead
#411	30.0	33.8	32.1	27.6	dead
#412	36.9	38.8	37.8	38.5	40.1
#413	33.8	37.2	36.8	37.1	39.2
#414	38.6	40.6	38.6	dead	dead
#415	32.5	37.7	36.1	36.5	35.6

Table 21A, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 5					
#416	35.4	39.3	38.5	40.1	47.1
#417	33.8	38.2	39.9	40.2	41.3
#418	30.2	32.2	32.0	32.2	35.7
#419	35.2	36.4	35.7	35.1	37.2
#420	31.7	34.0	34.9	35.3	39.7
#421	38.6	40.7	41.0	41.1	47.1
#422	39.4	40.6	40.3	39.6	41.3
#423	35.8	37.5	37.1	36.8	41.5
Group 6					
#424	32.9	33.9	31.9	dead	dead
#425	37.9	40.3	38.4	36.4	dead
#426	37.7	39.9	38.9	38.3	41.0
#427	31.9	33.4	32.7	30.9	dead
#428	31.7	33.1	32.3	32.3	33.2
#429	42.2	45.1	45.8	45.4	46.8
#430	31.6	32.4	31.8	32.3	36.3
#431	34.5	38.1	37.5	37.9	42.9
Group 7					
#432	42.3	45.6	45.7	44.3	dead
#433	35.9	37.2	36.5	37.5	41.7
#434	37.5	42.7	41.1	39.0	dead
#435	42.9	43.7	42.3	42.9	51.7
#436	34.6	35.3	34.0	32.5	32.6
#437	35.6	36.6	37.1	36.7	40.6
#438	33.0	34.8	35.8	36.8	41.3
#439	35.9	36.9	34.0	dead	dead
Group 8					
#440	33.6	35.2	38.8	43.1	50.4
#441	33.3	37.1	38.6	42.3	49.8
#442	33.4	36.0	38.0	40.4	43.9
#443	33.5	36.5	38.4	37.6	40.7
#444	41.2	44.4	47.3	49.2	51.7
#445	34.1	38.5	41.0	44.2	48.2
#446	34.5	37.2	37.8	39.2	44.5
#447	35.5	38.5	39.1	42.0	49.5

Table 21A, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 9					
#448	41.1	40.7	39.9	36.7	dead
#449	36.6	38.3	38.1	37.7	38.8
#450	36.0	37.4	36.9	36.4	dead
#451	31.7	32.4	32.8	31.9	32.3
#452	32.3	34.0	33.7	32.7	33.6
#453	33.2	35.2	34.8	33.5	34.2
#454	33.9	35.2	35.2	34.3	35.2
#455	38.0	40.3	39.7	39.3	39.4
Group 10					
#456	40.4	42.5	45.0	43.2	44.1
#457	35.2	36.9	35.6	36.0	36.9
#458	37.2	42.8	41.7	39.1	dead
#459	40.2	41.5	41.1	40.1	dead
#460	38.5	41.0	41.2	40.2	44.8
#461	30.5	31.5	31.6	32.5	31.4
#462	36.1	38.6	39.1	38.0	40.0
#463	32.6	37.0	36.4	35.6	36.9
Group 11					
#464	39.9	42.2	41.8	40.3	40.5
#465	34.7	36.6	36.9	38.4	37.4
#466	31.3	32.3	32.0	dead	dead
#467	34.4	35.8	36.4	35.8	36.0
#468	36.1	39.5	38.5	38.2	dead
#469	37.8	40.8	40.5	40.5	39.4
#470	30.0	32.8	33.6	33.3	34.7
#471	32.6	34.7	34.8	35.2	35.9
Group 12					
#472	38.0	40.0	40.9	40.8	38.7
#473	32.8	38.1	37.3	38.5	37.6
#474	39.9	42.8	44.6	44.9	46.9
#475	32.7	35.9	37.2	38.0	38.6
#476	38.2	41.9	43.1	42.9	43.9
#477	34.7	38.6	41.2	41.7	41.4
#478	36.8	41.1	40.8	39.9	40.0
#479	35.5	38.7	39.1	40.0	38.4
Group 13					
#480	29.3	37.3	45.8	51.8	63.1
#481	28.6	28.1	dead	dead	dead
#482	36.0	45.1	55.9	69.6	75.3

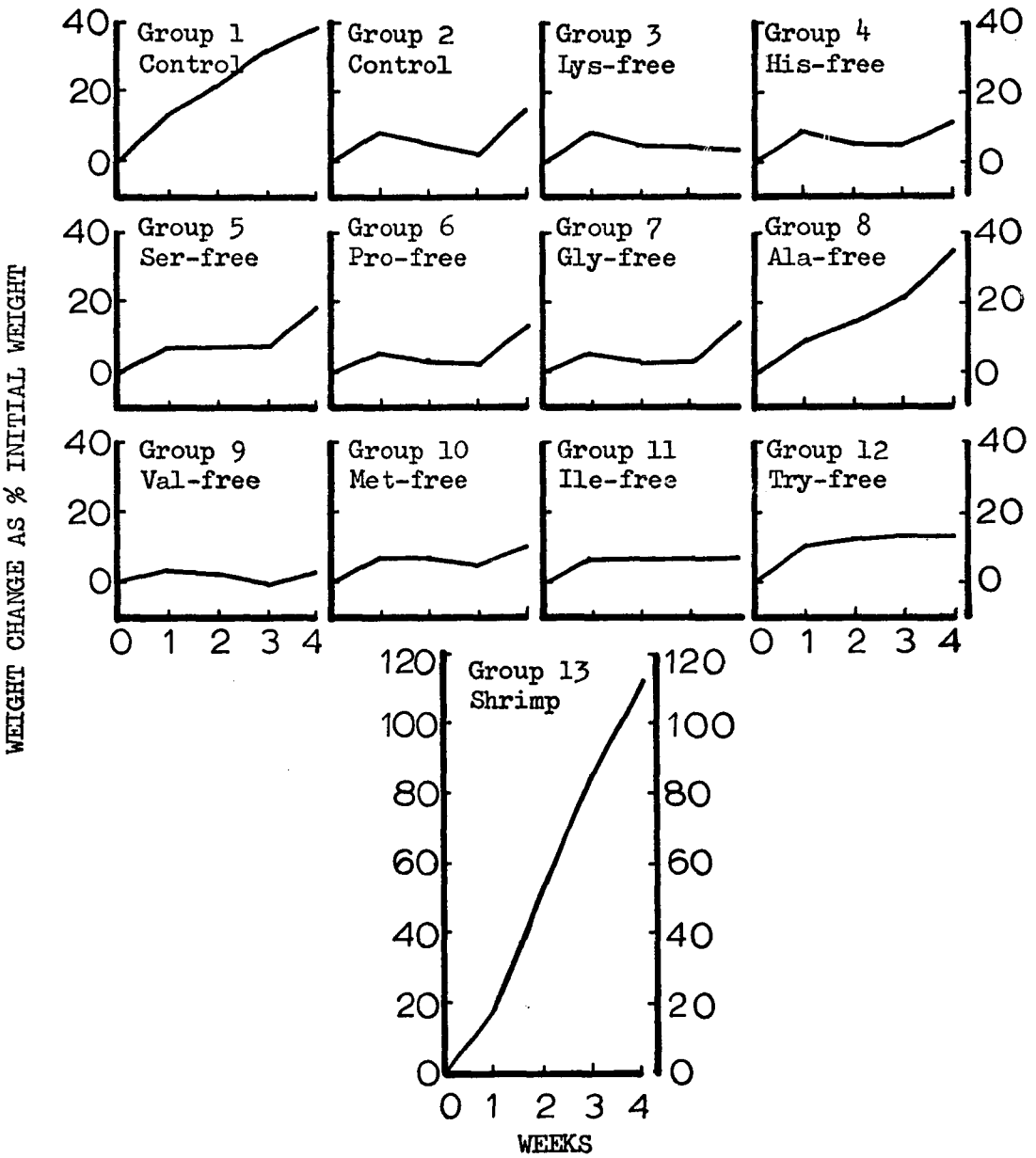


Figure 7A. Average weight change of turtles in each group of Experiment 11 as a percentage of their initial weight

(L-alanine-free amino acid mixture) were losing weight following modest gains during the initial week. Why Groups 1 and 8 did not decline in weight as did the other groups could not be determined. Average diet consumption/turtle/week for all groups receiving the synthetic diets was 17.4 grams for week 1, 23.7 grams for week 2, and 23.5 grams for week 3. Food consumption for turtles in Group 1 was only slightly higher than the average values; however, food consumption values for Group 8 were approximately 1.5 times average at 19.8 grams, week 1; 34.1 grams, week 2; and 34.8 grams, week 3. Although a few deaths had occurred, it did not seem that disease was a major factor in the failure of the groups to gain weight as expected. Since results at the end of the third week indicated that the experiment was going to fail in meeting its objective, I decided to modify the diets. All the groups were ingesting relatively large amounts of food (approximately 8-10% of their weight per day). It was felt that the poor utilization of the ingested diet was possibly the factor responsible for poor weight gains. I decided to increase the level of amino acid mixture in the synthetic diets from 18% to 36% wet weight and at the same time reduce the level of potato starch from 20% to 10% of the diet. The rationale for this was that perhaps the amino acid component was more readily utilized than was the starch component. If this were the case, it was possible that the hatchlings were not getting sufficient energy for growth from the diet. By increasing the amino acid level it was hoped that this protein component would provide both amino acids for protein synthesis and energy for metabolic functions. The level of

starch was decreased due to the possibility that the starch was making much of the diet unavailable for absorption by the digestive tract.

The turtles were fed these modified diets during the last week of the experiment. As can be seen from Figure 7A, several of the groups showed a very positive response to the increased protein level. The groups which failed to show this positive response were Group 1, control diet based on shrimp amino acid pattern; Group 3, L-lysine-free diet group; Group 4, L-histidine-free diet group; Group 9, L-valine-free diet group; Group 10, L-methionine-free diet group; Group 11, L-isoleucine-free diet group; and Group 12, L-tryptophan-free diet group. With the exception of Group 1, all these groups were fed diets lacking traditional nutritionally essential amino acids and, therefore, could not logically be expected to respond to increased levels of a severely deficient protein source, if the nutritionally essential amino acids required by the hatchling sea turtle are in fact those required by most other animals.

The experiment was terminated at the end of the fourth week for two reasons: (1) the increased level of amino acids used in these diets made it necessary to return to Tucson to obtain additional supplies of amino acids and (2) a new shipment of hatchlings had arrived in Tucson for the next experiment.

Experiment 12

Objective

This experiment to determine the nutritionally essential amino acids required by the hatchling Green sea turtle.

Materials and Methods

The C. mydas hatchlings were from eggs laid on nesting beaches in Surinam and hatched at Mariculture, Ltd., Grand Cayman Island. The turtles were fed shrimp during the 17 day period after arriving from Grand Cayman and prior to beginning the experiment. These turtles were transported from the Tucson laboratory to the Guaymas, Sonora laboratory. The turtles appeared to be in good physical condition but were almost twice the size of hatchlings used in the previous experiment, indicating that the turtles were approximately 4 weeks old and had been fed while at Mariculture, Ltd. The turtles were individually tagged and weighed and then divided into 19 groups of 6 turtles each. The smaller number of turtles per group was used because the size of each turtle was so much greater than in the last experiment. The turtles were re-weighed at the beginning of each week.

The water system used in this experiment was similar to that used in Experiment 11. Hatchlings were kept in the same dish pans as before, except the size of the glass tubing used for a drain pipe was increased from 10 mm to 12 mm to provide more rapid draining. The water used differed from that in Experiment 11 in that it was filtered by passing it through cartridge-type filter elements and water temperature was maintained by using a heat-exchanger. The use of the heat

exchanger was necessary since ambient sea water temperature was now approximately 32°C. Water temperature was maintained between 26-27°C for most of the experiment; however, several power failures of short duration allowed water temperature to occasionally approach ambient sea water temperature. Water flow and cleaning procedures were the same as for Experiment 11.

The diets used were modified as discussed in the last experiment, with the level of amino acid mixture being 36% of the wet weight of the diet and with the amount of potato starch reduced to 10%. Group 1 received the control diet (Diet 085) which contained an amino acid mixture based upon the composition of casein. This diet contained all 18 test amino acids. Diets 086-103 were modifications of Diet 085; in each case, a single amino acid was deleted and the amount of removed nitrogen replaced using diammonium citrate. The test groups received the following diets: Group 2, Diet 086 (L-lysine-free); Group 3, Diet 087 (L-histidine-free); Group 4, Diet 088 (L-arginine-free); Group 5, Diet 089 (L-aspartic acid-free); Group 6, Diet 090 (L-threonine-free); Group 7, Diet 091 (L-serine-free); Group 8, Diet 092 (L-glutamic acid-free); Group 9, Diet 093 (L-proline-free); Group 10, Diet 094 (glycine-free); Group 11, Diet 095 (L-alanine-free); Group 12, Diet 096 (L-cystine-free); Group 13, Diet 097 (L-valine-free); Group 14, Diet 098 (L-methionine-free); Group 15, Diet 099 (L-isoleucine-free); Group 16, Diet 100 (L-leucine-free); Group 17, Diet 101 (L-tyrosine-free); Group 18, Diet 102 (L-phenylalanine-free); and Group 19, Diet 103 (L-tryptophan-free). The composition of Diet 085 and the amount of

diammonium citrate used to replace each deleted amino acid is given in Table 1 (p. 14 in dissertation text).

Diet preparation was the same as in previous experiments, but the feeding method was changed. The addition of more amino acid mixture to the synthetic diets, even with the reduction of starch, produced a diet with a less fluid consistency than synthetic diets used in previous experiments (with the exception of the diets used the last week in Experiment 11). The mixtures could be forced into the glass tubes only with great difficulty. It was also very difficult to force the diets out of the tubes for feeding, this procedure often resulting in broken tubes and cut fingers. For these reasons, it was decided to revert to the original feeding procedure used in the early experiments, that is, chunks of the diet were simply dropped into the container with the hatchlings. Diets were stored in wide-mouth jars which were kept in a refrigerator. Food consumption was determined by weighing the jars before feeding in the morning and re-weighing the jars in the evening after feeding. Uneaten food was removed from the tubs and weighed. The turtles were fed 6 days each week, not being fed the day prior to weighing.

Results and Interpretation

From Table 22A, which gives the weights of each hatchling during the experimental period, it can be seen that most groups had suffered severe losses due to disease by the end of the fourth week. Total mortality was 68%. Figure 8A shows the average weight change as percent of initial weight for each group. The value for each week was calculated

Table 22A. Individual weights in grams of turtles in each group of Experiment 12 during experimental period

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 1					
#505	57.7	57.9	61.9	dead	dead
#566	50.3	51.2	46.6	47.5	dead
#567	57.8	58.5	54.2	dead	dead
#580	45.4	47.0	47.6	47.6	46.9
#598	54.0	47.7	43.8	dead	dead
#609	54.0	49.3	dead	dead	dead
Group 2					
#509	49.1	48.3	49.4	dead	dead
#529	49.4	48.1	47.8	dead	dead
#533	50.7	43.0	dead	dead	dead
#552	47.5	dead	dead	dead	dead
#561	45.2	42.7	dead	dead	dead
#619	43.5	45.6	45.7	45.8	46.4
Group 3					
#513	43.9	43.2	dead	dead	dead
#546	44.9	dead	dead	dead	dead
#548	47.7	dead	dead	dead	dead
#595	45.0	40.7	dead	dead	dead
#605	48.0	43.4	dead	dead	dead
#623	54.3	51.7	54.2	55.3	53.5
Group 4					
#489	45.2	37.5	dead	dead	dead
#511	44.3	41.9	dead	dead	dead
#515	47.4	dead	dead	dead	dead
#550	54.9	52.2	53.0	53.7	55.6
#581	56.4	55.3	56.1	dead	dead
#628	43.9	41.5	dead	dead	dead
Group 5					
#556	50.9	45.8	46.4	47.5	47.1
#560	53.6	41.6	dead	dead	dead
#569	55.5	52.5	48.5	43.6	dead
#602	51.8	47.1	47.0	45.2	dead
#607	44.6	41.1	40.4	37.4	dead
#610	49.3	42.9	36.8	dead	dead

Table 22A, Weights of turtles in Experiment 12, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 6					
#551	52.6	50.6	48.6	45.6	46.9
#557	46.8	44.5	43.4	dead	dead
#571	49.5	dead	dead	dead	dead
#573	54.0	53.0	55.4	55.9	dead
#576	45.8	42.5	40.5	44.6	dead
#625	61.6	dead	dead	dead	dead
Group 7					
#483	54.7	54.6	58.0	59.8	66.5
#512	52.0	dead	dead	dead	dead
#514	43.8	dead	dead	dead	dead
#530	47.0	43.1	37.4	dead	dead
#577	50.6	52.5	57.2	63.1	59.2
#636	57.2	55.1	59.3	68.5	68.1
Group 8					
#522	45.0	41.4	44.5	46.9	38.9
#531	53.9	49.5	49.6	57.6	65.7
#538	44.8	42.0	41.2	42.6	dead
#565	60.6	53.8	52.5	dead	dead
#600	43.0	dead	dead	dead	dead
#630	45.7	38.1	dead	dead	dead
Group 9					
#485	44.1	49.2	52.6	57.0	59.2
#497	46.0	49.9	53.9	57.5	63.9
#521	45.9	47.4	52.5	55.2	59.4
#572	46.4	46.5	48.2	48.6	48.4
#599	45.3	43.7	dead	dead	dead
#626	52.0	53.7	57.8	61.9	67.4
Group 10					
#518	43.6	35.8	dead	dead	dead
#585	52.5	53.2	dead	dead	dead
#587	55.3	62.1	70.8	78.9	88.9
#588	46.9	49.0	54.5	52.3	dead
#591	46.6	48.1	50.9	dead	dead
#634	45.2	42.9	46.6	47.5	dead

Table 22A, Weights of turtles in Experiment 12, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 11					
#527	53.4	58.6	64.9	73.2	83.2
#574	44.3	44.1	51.8	54.9	55.2
#612	57.5	63.6	71.9	80.0	90.6
#620	48.1	48.9	50.2	54.8	60.3
#622	46.9	47.4	49.1	51.2	dead
#631	50.6	53.7	57.6	61.5	60.8
Group 12					
#493	55.6	58.8	66.5	74.7	74.5
#539	51.3	52.3	56.6	63.0	71.3
#553	58.6	64.2	66.6	76.6	76.7
#559	50.6	52.1	56.1	62.5	dead
#564	46.9	dead	dead	dead	dead
#592	44.8	44.8	48.6	51.1	55.3
Group 13					
#486	44.6	44.4	dead	dead	dead
#487	50.1	48.3	46.9	39.8	dead
#501	51.6	49.0	50.0	dead	dead
#537	51.7	50.7	52.6	dead	dead
#597	52.4	50.3	48.3	48.6	dead
#601	61.4	59.2	62.2	61.5	60.8
Group 14					
#530	52.2	47.6	50.8	45.8	dead
#543	58.3	55.0	dead	dead	dead
#584	59.3	53.8	dead	dead	dead
#594	46.9	45.3	44.9	dead	dead
#611	42.5	40.4	dead	dead	dead
#624	46.6	43.9	dead	dead	dead
Group 15					
#495	53.6	50.6	48.7	dead	dead
#523	46.6	38.3	dead	dead	dead
#562	45.9	42.3	42.3	dead	dead
#608	42.9	40.3	38.3	36.0	dead
#615	51.0	47.2	48.2	45.9	48.6
#633	45.1	45.8	40.6	dead	dead

Table 22A, Weights of turtles in Experiment 12, Continued

Group	Initial	Week 1	Week 2	Week 3	Week 4
Group 16					
#510	50.1	48.5	46.9	dead	dead
#545	57.9	45.5	46.3	dead	dead
#554	54.8	54.5	57.5	58.1	58.4
#582	45.0	38.0	dead	dead	dead
#603	53.3	46.9	dead	dead	dead
#629	50.5	45.5	45.5	44.0	47.1
Group 17					
#488	52.7	55.2	55.4	65.8	75.6
#502	53.8	58.9	64.1	69.6	70.4
#525	48.8	52.8	59.7	60.4	dead
#568	54.8	57.4	59.9	dead	dead
#578	54.9	53.7	58.8	62.3	67.9
#583	54.2	55.2	61.0	66.4	dead
Group 18					
#526	44.7	43.6	43.2	42.9	dead
#532	49.2	45.9	46.6	48.1	50.6
#547	47.5	45.9	47.6	44.2	dead
#549	48.9	46.3	47.0	46.4	46.0
#621	53.4	51.4	52.6	53.0	53.0
#635	44.2	40.3	dead	dead	dead
Group 19					
#484	47.5	47.7	47.5	47.7	47.7
#499	43.2	42.2	42.8	36.9	dead
#542	45.0	42.7	dead	dead	dead
#589	48.6	45.1	42.2	dead	dead
#606	49.4	dead	dead	dead	dead
#618	55.6	49.8	dead	dead	dead

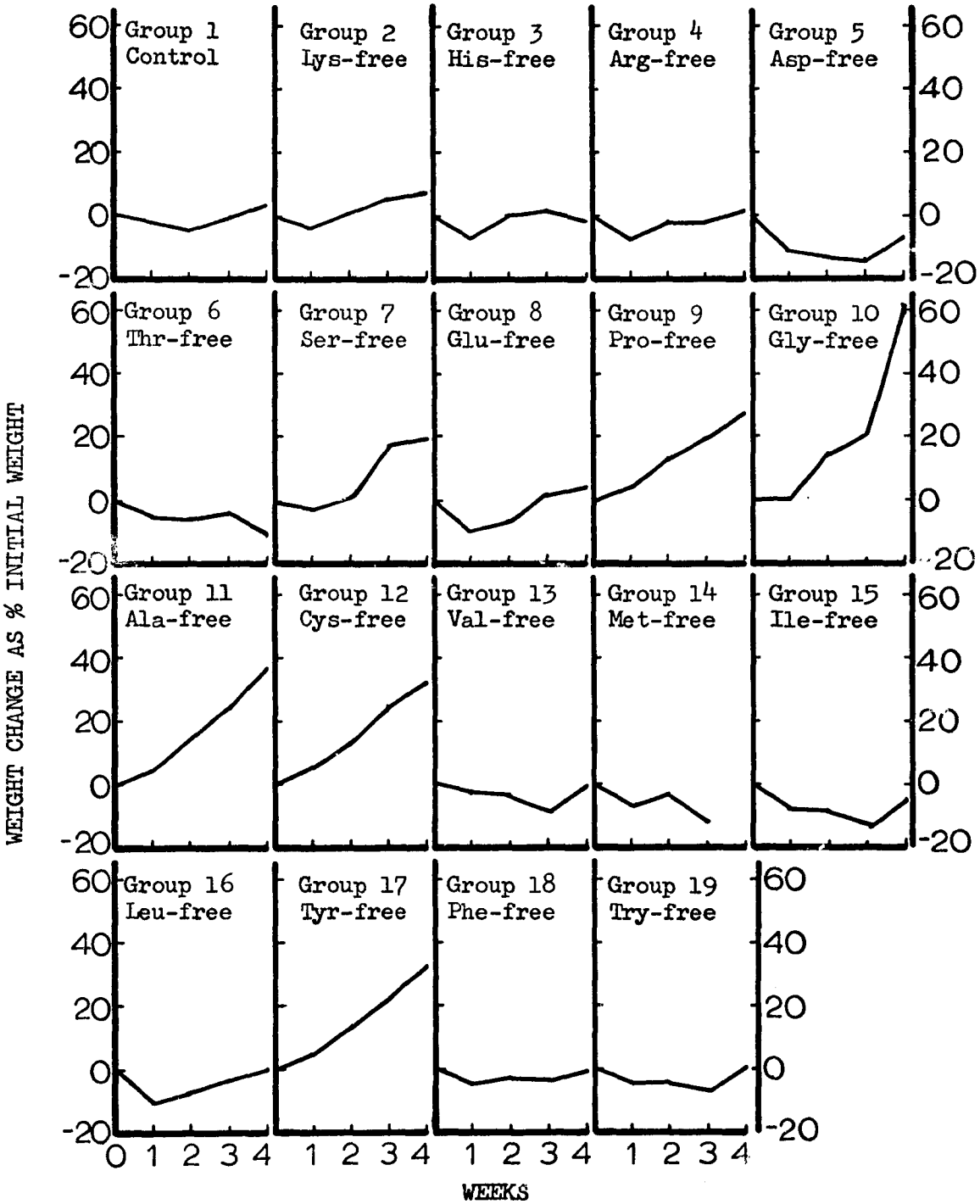


Figure 8A. Average weight change of turtles in each group of Experiment 12 as a percentage of their initial weight

by determining the weight change as percent of initial weight for each hatchling surviving in the experimental group at that time, then averaging the values. From both Table 22A and Figure 8A it can be seen that most of the groups were virtually eliminated and survivors had ceased to grow by the fourth week. Since one of the groups hardest hit by the disease problem was the control group, Group 1, it was impossible to draw any conclusions from the experimental results as to essential amino acids. There were, however, 6 groups which had done relatively well, gaining weight, and for the most part, suffering less mortality than the other groups. These 6 groups were Groups 7, 9, 10, 11, 12, and 17. These groups received diets lacking either L-serine, L-proline, glycine, L-alanine, L-cystine, or L-tyrosine. While it was not conclusive, the results were interpreted to indicate that the 6 above-mentioned amino acids were nutritionally non-essential for the young Green sea turtle.

In a telephone conversation with a member of the staff of Mariculture, Ltd. it was learned that Mariculture, Ltd. had lost large numbers of the same group of hatchlings that had been used to provide turtles for this experiment. The deaths at Mariculture, Ltd. were apparently caused by an outbreak of coccidiosis. It seems probable that much of the mortality experienced in this experiment was due to this same disease, carried with the turtles from Grand Cayman Island.

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