Nutritional Benefit of a Marine Animal Gelatin Diet as Measured by Sea Turtle Blood Chemistry Values

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INTRODUCTION

The use of unflavored animal gelatin as a binding agent for marine animal food mixtures is not a new approach in aquarium animal husbandry. Professional aquarists have utilized gelatin diets for many years as an approach to offer a nutritionally balanced, and yet economically and easily prepared aquarium animal diet (Klocek, 1982; Peterson et. al., 1967; Sciarra, 1976; Spotte, 1973; Spotte et. al., 1985). Gelatin-bound diets for aquatic animals were utilized even earlier by researchers involved with establishing the nutritional requirements of a few economically important species of fishes (salmonids, catfish, etc.) used in aquaculture applications (Halver, 1957; 1972). More recently, research has begun on the nutritional requirements, digestive physiology, and diet formulations for sea turtles. This, however, has been necessarily restricted to the commercially important green turtle, Chelonia mydas, which as an adult is exclusively herbivorous (Bjorndal, 1985). With the exception of research on these few commercially valuable species, very few quantitative results have been reported from the use of these diets. Indeed, most of what exists in the husbandry or hobbyist literature is purely qualitative (species lists of those animals which readily consume the diet), or is simply reporting new preparative techniques. Those that do present quantitative results do so in forms ranging from growth rates for cultured species, to pigmentation enhancement in aquarium fishes (Boonyaratpalin and Lovell, 1977; Fey and Meyers, 1980) neither of which is a true indication of nutritional benefit or of animal health.

Until relatively recently, captive marine animals held for display or research purposes, have been maintained on natural foods. The idea, of course, being that since these foods form part of the diet in the wild, they should provide a complete diet. This premise, however, fails to take into consideration a number of important factors, the most important being that the nutritional requirements of animals in captivity are significantly different than those in the wild (Frye, 1981). Additionally, the components of exotic animal's natural diet may not always be available.

The advent of aquaculture, and it's associated economics, has led to the investigation of the nutritional requirements of various captive species of fishes and reptiles. Although there are marked species differences in regard to these nutrient requirements, the results from these aquacultural studies offer sufficient basic guidelines.

Thus, when designing an artificial diet suitable for a wide variety of captive marine animals, a number of factors need to be considered. Foremost among these is the type of animal being considered; carnivores, omnivores, or herbivores. Obviously one diet will not be nutritionally complete for all marine animals normally kept in captivity, however, most fishes and sea turtles are essentially opportunistic and, in captivity, omnivorous. Those animals which are strictly carnivorous or herbivorous can be dealt with separately.

Other important considerations in formulating a gelatin-bound aquarium animal diet include:

1) Stability - the diet should be relatively water stable, and resistant to crumbling in order to minimize waste and water contamination.

2) The relative concentrations of protein, fat, carbohydrates (fishes are not known to require this at all), and caloric content.

3) Vitamin content - many vitamins are known to be essential and their absence, or over abundance, can cause nutritional disorders. Additionally, when using frozen fish flesh as a component of a diet, the natural loss of thiamine (Vitamin B_1) must be compensated for.

4) Mineral content - Fish require large amounts of calcium and phosphorus for growth and development (Cowey and Sargent, 1979; Lovell, 1979; Post, 1978). Marine fish are able to obtain most of their calcium from the surrounding water, but phosphorus must be supplied by their diet. In captive reptiles (and in this case, sea turtles) the ratio (serum or plasma) of calcium to phosphorus (Ca:P) is of major clinical significance (Frye, 1981). The literature generally recommends a Ca:P ratio for reptiles from 1.2:1 (Fraser and Mays, 1986) to 1.5:1 (Frye, 1981; Moore, 1987). In some situations, such as females laying large numbers of calcareous eggs, or in rapidly growing juveniles, a Ca:P ratio approaching 2:1 is recommended (Fraser and Mays, 1986). A deficient Ca:P ratio (less than unity) may result in nutritional secondary hyperparathyroidism, fibrous osteodystrophy, osteomalacia, pathological fractures, bone deformities, or soft or deformed shells (Fraser and Mays, 1986). The condition may be compounded by a Vitamin D deficiency. Vitamin D (specifically D_3 in reptiles) is involved in calcium metabolism and distribution. Obviously, since sea turtles possess so much skeletal material, the levels of calcium and phosphorus are of prime importance in their diet.

We report a quantitative comparison of sea turtle blood chemistry values (specifically calcium and phosphorus) between turtles initially fed a "natural" diet (crabs, fish and shrimp) which developed dangerously imbalanced calcium to phosphorus ratios (Ca:P), and turtles which were fed a marine animal gelatin diet exclusively.

METHODS

1) Animal Husbandry

The animals used in this study were six (6), one-year-old loggerhead sea turtles, Caretta caretta, which were raised from hatchlings at the

Virginia Institute of Marine Science (VIMS). Five of the yearling loggerhead sea turtles (#1-5) were kept inside of a temperature-controlled greenhouse in a single 480 gallon (1,817 liter) fiberglass tank, divided into five equal compartments. Another one of these yearling loggerhead turtle (#6) was kept on display in a 180 gallon (681 liter) fiberglass tank in the VIMS Aquarium. A seventh turtle of the same approximate age group, but from a different clutch and geographical origin, was later added to the study as a means of confirmation. This turtle (#7) was on loan to the Virginia Living Museum, Newport News, VA, from the Florida Department of Natural Resources, Naples, FL.

All of the turtles were kept in recirculated (closed) artificial seawater systems maintained at a salinity range of 28-35 ppt and at a temperature range of 26^{0}-30^{0}C. The turtles were maintained in accordance with the "Care and Maintenance Standards for Sea Turtles Held in Captivity" specified by the Federal Wildlife Permit Office, and under the guidelines set forth for the care of sea turtles in captivity by Pritchard et. al. (1983).

Initially, turtles #1-5 were fed a "natural" diet. The natural diet of loggerhead turtles is rather catholic and includes fishes, barnacles, shrimp, crabs (blue, spider, rock, and horseshoe), squid, mollusks, sponges, jellyfish, and even sea grasses (Bellmund et. al., 1987; Lutcavage and Musick, 1985; Musick, 1979). In this case, the "natural" diet consisted of shrimp (Penaeus sp.), squid (Loligo sp.), blue crab (Callinectes sapidus), rock crab (Cancer irroratus), and fishes (Leiostomus xanthurus, Micropogonias undulatus).

Turtle #6 was fed the VIMS Aquarium gelatin diet (VGD) exclusively. After it was discovered, by examination of blood chemistry values, that turtles #1-5 had disproportionate Ca:P ratios, they were switched over to the VGD exclusively. Turtle #6 had an average pre-study Ca:P ratio of 1.15:1, which is within the recommended normal range. Thus, it was hypothesized that perhaps the VGD was responsible for creating a healthier nutritional state in captive juvenile loggerhead sea turtles. After some initial post-diet change data were collected, the seventh loggerhead yearling was added to the study and switched from a "natural" diet to the VGD supplied by VIMS.

All turtles, regardless of diet, were fed daily ad libatum.

2) Gelatin Diet

The gelatin diet used in this study is a modification of the diet described by Choromanski (1985) which was also a modification of the gelatin diet reported by Sciarra (1976).

The VIMS Aquarium gelatin diet (VGD Table 1) was designed to include the widest variety of ingredients with markedly different chemical and nutritional compositions, in order to include adequate levels of as many naturally-occurring nutritional components as possible. This was accomplished by referring to the vast food composition tables of Watt and Merrill (1950), Orr and Watt (1957), and Sidwell (1981). By comparison of relative values, food components were empirically chosen so that the overall

levels of protein, lipid, specific minerals or vitamins could be coordinated. For example, four 5 oz. portions of different species of raw fish were used with comparatively different compositions (Table 1 & 2) rather than just one 20 oz. fish component. Cod (Gaddus morhua) was chosen for its relatively high thiamine levels, smelt (Osmeridae) for the calcium and phosphorus levels, carrots for high Vitamin A, etc.

Proximate analysis of the VIMS Aquarium gelatin (VGD) has not been performed to date, however, a reasonable estimate can be gained by comparing the percent composition of the VGD (Table 1) to the chemical and nutritional composition of the various ingredients (Table 2).

Amino acid and vitamin supplements were added to fill in deficiencies inherent in the natural foods. For example, components such as Vitamin D_3 were added, which is essential for captive reptiles (Frye, 1981).

3) Blood Chemistry

Blood was taken from the turtles for constituent analysis as often as possible, however, due to their relatively small size, and presumed low blood volume, a minimum sample interval of six weeks was deemed prudent.

Two ml blood samples were obtained from each turtle utilizing the dorsal cervical sinus bleeding technique (Owens and Ruiz, 1980). Blood samples were transferred from syringes to Lithium heparinized "Vacutainers", and stored temporarily at 0-4^{0}C until analyzed.

Blood was analyzed by Riverside Veterinary Diagnostic Services (Walter Reed Memorial Hospital, Gloucester, VA) utilizing a Hitachi 705 Blood Analyzer. Specifically, the minerals in question were determined spectrophotometrically via an ammonium molybdate reaction with absorbance measured at a wavelength of 340 nm for phosphorus, and via a o-cresophthalein complexone reaction with absorbance measured at wavelength of 660 nm and 570 nm.

RESULTS

The yearling loggerhead sea turtle, which was fed the VIMS Aquarium gelatin diet (VGD) exclusively (#6), had a pre-study mean plasma Ca:P ratio of 1.15:1, a post-study mean ratio of 1.30:1, with an overall mean Ca:P ratio of 1:24:1 (Figure 1).

Turtles #1-5 all possessed dangerously low (well below 1.0:1) plasma Ca:P ratios while being fed a natural diet, but improved dramatically after being switched to the VGD exclusively. The trend of the change of Ca:P ratios for turtles #1-5 was remarkably similar (Figures 1 and 2) and represents an overall (combined means for turtles #1-5) change from a pre-diet mean plasma Ca:P ratio of 0.49:1, to a post-diet mean Ca:P ratio of 1.48:1.

Turtle #7 also had a low pre-diet Ca:P ratio, but not nearly as critical as turtles #1-5. The trend of the ratio change for turtle #7 was somewhat different as well, although steadily increasing to safe levels, the values did not fluctuate as much as they did for turtles #1-6 (Figure 2).

Turtle #7 had a pre-diet Ca:P ratio of 0.90:1, but increased to a post-diet mean Ca:P of 1.38:1 (Figure 1).

These changes in the plasma Ca:P ratio resulting from a change from a natural diet to the VGD were significant (Student's T-test $p < 0.05$).

DISCUSSION

The initial dangerously low plasma Ca:P ratios for turtles #1-5 was presumed to be caused by their being fed a "natural" diet of shrimp, crabs and fishes which, overall, have a much higher phosphorus content than calcium (Sidwell, 1981). It is presumed that in the wild this natural diet is not a problem because the loggerhead turtle's diet is augmented with a wider variety of foods including seagrasses. Additionally, wild sea turtles are exposed to sufficient u.v. radiation (sunlight) to satisfy their Vitamin D requirements. It is only under the stressful conditions produced by captivity that these, and other, nutritional and chemical disorders manifest themselves. Consequently, aquarists must compensate for this by offering diets with great variety of composition and supplementation to achieve an over abundance of the nutritional requirements.

Some of the later post-diet Ca:P ratios at first appear to be rather high, however, it must be remembered that the turtles used in this study were juveniles, still rapidly growing at one year of age, and actually require a relatively higher Ca:P ratio (Fraser and Mays, 1986).

It is interesting to note that even one day old loggerhead hatchlings in captivity will readily accept this diet. Also, although no measurements were taken, it should be noted that turtle #6, which was fed the gelatin diet for a longer period of time, grew faster and larger than the other yearlings from its cultch.

The fishes on display at the VIMS Aquarium (indigenous to Chesapeake Bay and off-shore mid-Atlantic region waters) all readily feed on the gelatin as well.

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TABLE 1A. VIMS AQUARIUM GELATIN DIET

INGREDIENT	oz.	g	%	Cost/lb. ($)	Portion Cost ($)
1. TROUT CHOW (Purina #5112 sinking)	30	850	15.6	0.30	0.56
2. FISH (raw, frozen):	20	565	10.4		
a) Cod (Gadus morhua)	5	141	2.6	3.12	0.98
b) Haddock (Melanogrammus sp.)	5	141	2.6	2.00	0.94
c) Smelt (Osmeridiae)	5	141	2.6	3.00	0.94
d) Whiting (Menticirrus sp.)	5	141	2.6	1.10	0.34
3. SQUID (raw, frozen) (Loligo brevis)	10	285	5.25	1.10	0.69
4. SHRIMP (raw, frozen) (Penaeus sp.)	10	285	5.25	1.10	1.36
5. SPINACH (raw, frozen)	10	285	5.25	0.43	0.27
6. CARROTS (raw, frozen)	10	285	5.25	0.42	0.26
7. SUPPLEMENTS					
a) A.A. 1000[1]	0.14	4 (ml)	0.07	14.00 (l)	0.06
b) SEA TAB vitamins[2]	0.14	4	0.07	0.05(each)	0.20
8. WATER	81.1	2400(ml)	44.2	-	-
9. UNFLAVORED ANIMAL GELATIN (Vyse Gelatin, 300 bloom)	16	450	8.3	2.50	2.50
	TOTAL BATCH COST				$9.10

-1- A.A. 1000
(Beecham Laboratories, Bristol,TN)
- each 100 ml contains the following, pure crystalline amino acids, vitamins, electrolytes, and dextrose: Valine (136 mg); Leucine (187 mg); Isoleucine (85 mg); Arginine-HCl (85 mg); Histidine-HCl H_2O (59.5 mg); Methionine (51 mg); Phenylalanine (119 mg); Threonine (78.2 mg); Tryptophane (34 mg); Lysine HCl (170 mg); Pyridoxine HCl (10 mg); Cyanocobalamin (5 ug); Sodium Acetate (0.25 g); Calcium Chloride - $2H_2O$ (150 mg); Potassium Chloride (200 mg), Magnesium Sulfate - $7H_2O$ (200mg); Dextrose-H_2O (5 g).

-2- SEA TAB vitamins
(Pacific Research Laboratories, Inc. El Cajon, CA)
- each SEA TAB contains:
Vitamin A (12,500 I.U.); Vitamin D_3 (2,500 I.U.); Vitamin E (31.2 I.U.); Vitamin C (125 mg); Vitamin B_1 (250 mg); Vitamin B_2 (6.5 mg); Niacin (10 mg); Vitamin B_6 (3.75 mg); Vitamin B_{12} (15 ug); Panthotenic Acid (9.2 mg); Iron (25 mg); Choline, Inositol, Folic Acid, and Kelp (trace).

TABLE 1B. PROCEDURE FOR MAKING VIMS AQUARIUM GELATIN DIET.

1) Thaw all ingredients, and chop into small pieces.

2) In a 3 liter (or larger) pyrex bowl, heat 1/2 of the water (1200 ml) to 75^{o}-90^{o}C. Slowly add the powdered gelatin to this hot water and stir until all lumps are removed.

3) In a one gallon commercial blender (Waring), bring trout chow, fish, squid, shrimp, spinach, carrots and 1/2 of the water (1200 ml) to near liquid consistancy.

4) Add the gelatin mixture to the blended ingredients already in the blender, and add the nutritional supplements, and blend at high speed for 30 seconds.

5) Pour the mixture into 2 (13 x 8 1/2 x 2 inch) or 4 (8 1/2 x 8 1/2 x 2 inch) plastic freezer-tolerable containers. Place the containers into the refrigerator for 24 hrs. or until set and then they can be stored in the freezer until needed.

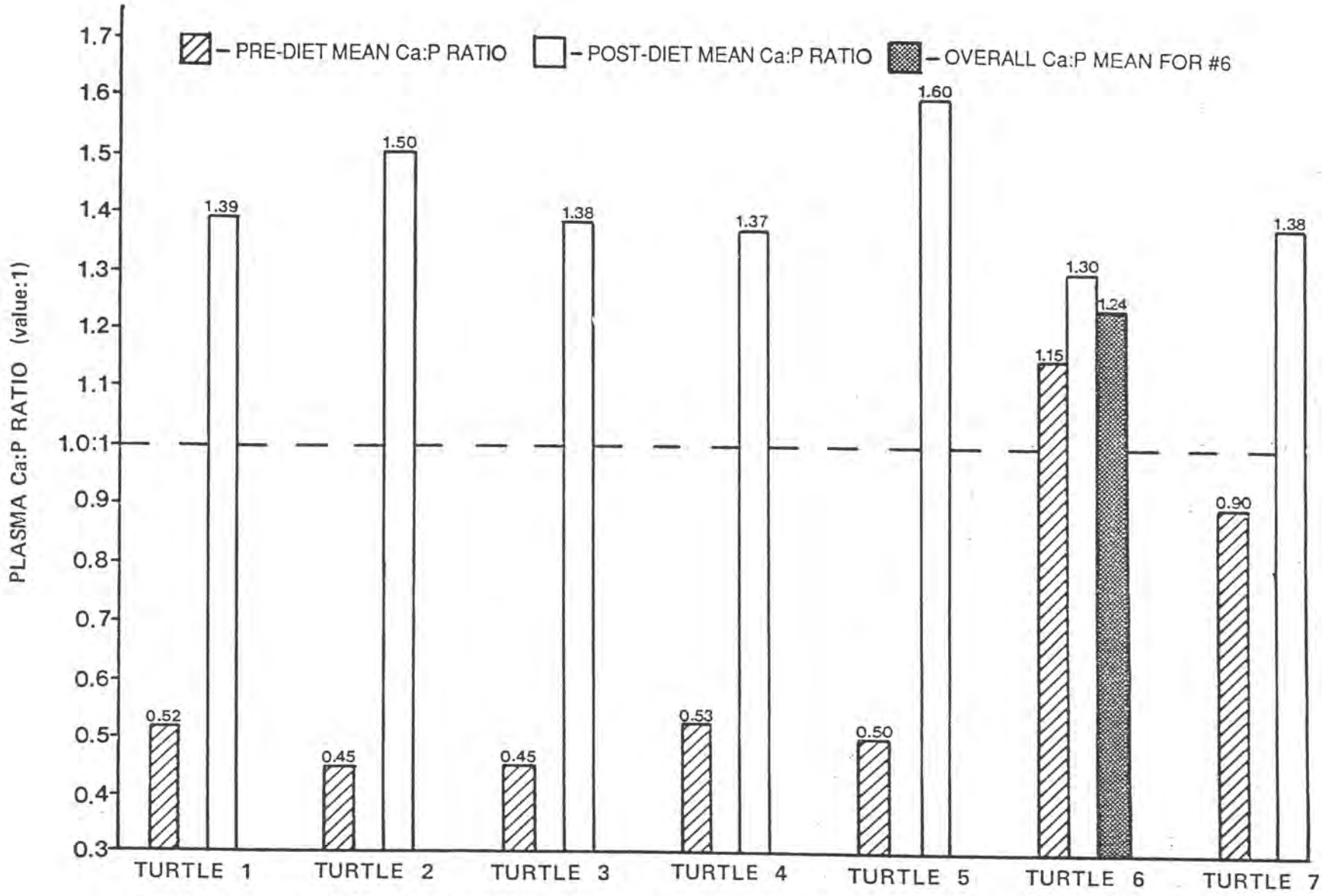
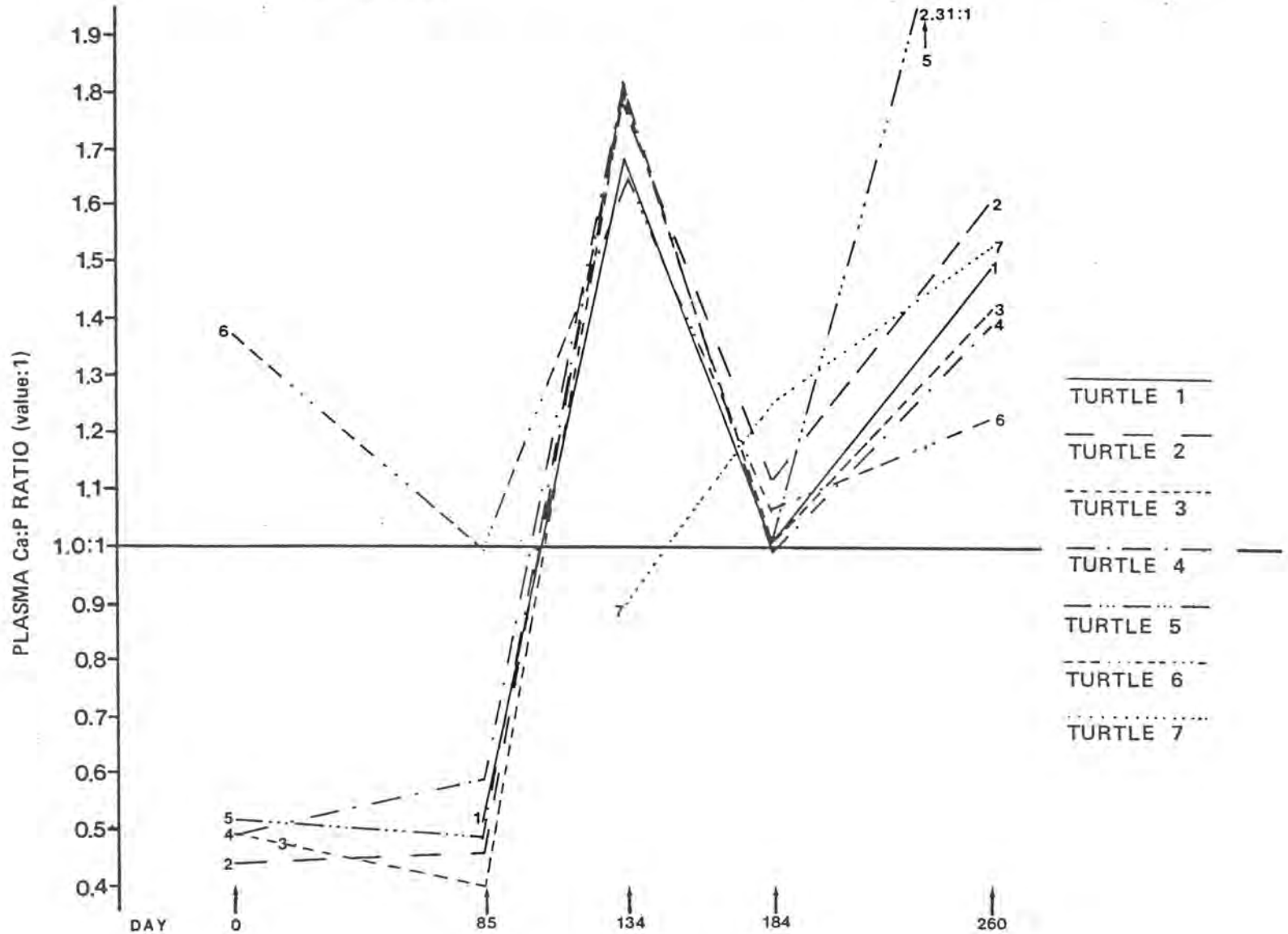


FIGURE 1. COMPARISON OF MEAN PLASMA Ca:P RATIOS IN YEARLING LOGGERHEAD SEA TURTLES, PRE- AND POST- GELATIN DIET.

FIGURE 2. PLAMSA Ca:P RATIOS OFF YEARLING LOGGERHEAD SEA TURTLES OVER TIME, PRE- AND POST-GELATIN DIET.



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