Effect of Polymethylolcarbamide (Urea Formaldehyde Condensation Polymer) on Growth and Tissue Formaldehyde Residues in Shrimp

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ABSTRACT

Toxicity of the urea formaldehyde resin pellet binder polymethylolcarbamide (Basfin^R) was determined in 28-day feeding trials conducted in tanks with *Penaeus vannamei* Boone, 1931. Levels of polymethylolcarbamide ranging from 0.25 to 8% did not affect shrimp survival. However, growth of shrimp fed feeds containing more than 0.5% polymethylolcarbamide was less than that of shrimp fed control feeds without polymethylolcarbamide. Growth was reduced 58% by feed containing 8% polymethylolcarbamide, and 19 to 27% by feeds containing 1 to 4% polymethylolcarbamide. In a separate trial, growth was reduced 29 and 30% by feeds containing 0.5 and 1% polymethylolcarbamide, respectively. Growth was reduced by polymethylolcarbamide both in feeds that were extruded without heating using alginate as the binder, and in feeds that were bound by polymethylolcarbamide with the addition of steam, heat and pressure. Formaldehyde residues in muscle increased linearly with polymethylolcarbamide level in feed. However, at levels of polymethylolcarbamide recommended by manufacturers for pellet binding, predicted levels of formaldehyde in shrimp tissues would be lower than those reported in stored fish and shrimp. Under conditions of semi-intensive pond culture where natural foods were present, a 28-day feeding trial conducted in outdoor pens indicated that growth and survival were not affected by 0.5% polymethylolcarbamide.

KEYWORDS: toxicity, pellet binder, feeding trial, *Penaeus vannamei*

Polymethylolcarbamide is a urea formaldehyde polymer that has been marketed by BASF Aktiengesellschaft under the trade name Basfin^R as a binder for pelleting mixed feedstuffs. It is also the major component of AQUA-TEC, marketed by Uniscope, Inc. as a pellet binder and waterproofing agent for use in fish and shrimp feeds, and of MAXI BOND, marketed by AGresearch, Inc. as a binder for all

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feeds. Manufacturer claims indicate that the addition of polymethylolcarbamide makes pelleted feeds harder, more durable, and more resistant to abrasion (BASF, 1983a). Increased stability of pellets in water due to polymethylolcarbamide has been reported in carp (BASF, 1983a) and in crustacean feeds (Boonyaratpalin, 1984; Uniscope, 1987, 1988a, 1988b, 1988c).

Although amounts of free formaldehyde in polymethylolcarbamide are relatively small, acid hydrolysis in the digestive system could release formaldehyde and urea. However, radio tracer studies with rats indicate that the primary path of polymethylolcarbamide elimination is fecal excretion and that renal and respiratory excretion are relatively minor (Grubenbecher and Kargarotos, 1983). This suggests that polymethylolcarbamide is excreted intact rather than metabolized, because the primary routes of elimination for free formaldehyde would be respiration and for free urea would be renal excretion. At the low rates that the binder is used in feeds, BASF (1983a) concludes that the urea and formaldehyde have no toxicological significance. Levels up to 0.5% in piglet feeds and up to 1% in broiler feeds, do not exert any adverse effects (BASF, 1983a).

Data describing the effects of polymethylolcarbamide on growth and survival have not been reported for penaeid shrimp. The objectives of this study were to (i) evaluate the effects of polymethylolcarbamide levels on the growth and survival of *Penaeus vannamei* in tanks, (ii) measure formaldehyde residues in shrimp tissues, (iii) determine if polymethylolcarbamide, when used under conditions similar to commercial applications, has adverse effects on growth and survival, and (iv) determine if effects of polymethylolcarbamide levels recommended for commercial feeds are present under conditions of semi-intensive pond culture where natural foods are additionally present.

MATERIALS AND METHODS

Three 28-day growth trials were conducted in indoor tanks where shrimp had access to dry feeds only. The first growth trial was designed to determine if high levels of dietary polymethylolcarbamide affect growth and survival, and to measure tissue levels of formaldehyde residues. Polymethylolcarbamide levels used in this trial were 0, 1, 2, 4, and 8%. The second growth trial was designed to determine the effects of dietary levels similar to those used in commercial feeds. Polymethylolcarbamide levels used in the second trial were 0, 0.25, 0.50, and 1.00%. The third growth trial was designed to determine if polymethylolcarbamide affected growth and survival when ingredients were conditioned at high temperature and pressure before extrusion. Levels of polymethylolcarbamide in the feeds were 0 and 1%. A fourth 28-day growth trial was conducted in pens within a pond where the shrimp had access to the substrate and natural foods as well as dry feeds. Polymethylolcarbamide levels used in the feeds for the pen study were 0 and 0.5%.

For the third growth trial, *Penaeus vannamei* postlarvae were obtained from Laguna Madre Shrimp Farms and grown to a suitable size. For all other growth trials, juvenile *P. vannamei* were obtained from the Texas A&M University Shrimp Mariculture Facility in Corpus Christi. For the first and second growth trials conducted in tanks, shrimp were acclimated for a minimum of 3 days before stocking. For the pond trial, which was conducted at the Texas A&M Mariculture Facility in Corpus Christi, the shrimp were stocked directly into the pens.

Table 1. Composition of feeds without polymethylolcarbamide. Values of ingredients and components represent percentages of diets on a dry weight basis.

Ingredient	Growth trial		
	1 & 2	3	4
Wheat starch†	21.3		21.1
Soybean meal‡	25.0	39.3	15.0
Wheat Middlings†		29.6	
Casein†	10.9		14.4
Soy protein (α-protein)†	10.9		14.4
Shrimp head meal§	6.2		5.0
Fish meal¶	6.2	20.0	5.0
AIN mineral mixture 76†	6.8	0.1	10.7
Calcium phosphate (dibasic)#		1.0	
Fish oil (menhaden)¶	3.9	1.0	5.5
Soybean lecithin (oil not removed)†	1.0	0.5	0.5
Cholesterol†	0.5	0.3	0.3
Vitamin mixture†,§§	2.0	2.6	2.0
Choline chloride#		0.1	
Ascorbic acid†	0.3		0.3
Ascorbic acid polyphosphate††		0.3	
Fish solubles¶	1.0		1.0
Cellulose (Alphacel)†	1.3	2.0	2.2
Sodium Alginate‡‡	2.0	2.0	2.0
Sodium hexametaphosphate#	1.0	1.0	1.0
Component			
Protein	40.0	40.4	40.0
Lipid	8.0	5.6	8.0
Fiber	4.0	4.1	4.0
Ash	13.0	10.3	15.0
Nitrogen free extract	35.0	39.6	33.0

†ICN Biomedicals, Inc., Costa Mesa, CA.
‡Producers Cooperative Association, Bryan, TX.
§Blum and Bergeron, Inc., Houma, LA.
¶Zapata-Haynie Corporation, Hammond, LA.
#VWR Scientific, Houston, TX.
††Vitamin Technologies International, Buhl, ID.
‡‡Kelco, Chicago, IL.
§§30 g/kg para-Amino benzoic acid (B_X), 1 g/kg D-Biotin (H), 1 g/kg Butylated hydroxylanisole, 1 g/kg Cholecalciferol (D_3, 400000 I.U./g), 75 g/kg Choline chloride, 1 g/kg Cyanocobalamin (B_{12}), 5 g/kg Folic acid (M), 180 g/kg Inositol, 2 g/kg Menadione (K_3), 26 g/kg Nicotinic acid, 15 g/kg D-Pantothenic acid (calcium salt), 3 g/kg Pyridoxine hydrochloride (B_6), 2 g/kg Retinyl palmitate (A), 8 g/kg Riboflavin (B_2), 498 g/kg Sucrose, 5 g/kg Thiamine mononitrate (B_1), 22 g/kg DL-alpha-Tocopherol acetate (E, 250 I.U./g).

Formulations of feeds and calculated proximate analyses are shown in Table 1. All control formulations without polymethylolcarbamide were complete feeds that were known from previous growth trials to produce satisfactory growth and survival of *Penaeus vannamei* in tanks without other supplemental feeds. The 1% polymethylolcarbamide feed used in the third growth trial was made by substituting 1% cellulose and 1% polymethylolcarbamide for 2% alginate in the control formulation. In the other growth trials, levels of polymethylolcarbamide were varied by substituting polymethylolcarbamide for equal amounts of wheat starch in the control formulations.

In commercial applications, the binding characteristics of polymethylolcarbamide are activated by the addition of steam and heat to the feed mix (BASF, 1983a). However, heat and pressure treatment of feeds containing the higher levels of polymethylolcarbamide used in the first growth trial (up to 8%) would produce very hard feeds that are unpalatable to shrimp. To reduce differences in water stability of the feeds due to the level of polymethylolcarbamide, all feeds, with the exception of the 1% polymethylolcarbamide feed used in the third growth trial, were prepared with alginate as a binder and extruded without heating (Meyers, 1980). These feeds were extruded through a 3.2 mm diameter die and dried at 50 to 60°C to a water content of less than 10%. The 1% polymethylolcarbamide feed used in the third growth trial was pelleted by Daishowa Chemicals, Inc. Ingredients used in the 1% polymethylolcarbamide feed were conditioned for 20 to 30 sec in a pellet conditioning chamber by heating with steam at 207 mla (30 psi) to 88°C. This feed was extruded through a 3.2 mm diameter die, held for 10 min without cooling, and cooled in a forced air drying oven to room temperature. All dried feeds were broken to an appropriate length (3 to 10 mm) and stored at -10°C.

The first two tank trials were conducted in 0.23 m^2 tanks containing 136 L of seawater. The third tank trial was conducted in 0.06 m^2 tanks containing 17 L of seawater. In the three tank trials, seawater was recirculated through tanks from a 58,500 L seawater system at respective rates of 83, 167, and 70% of tank volumes per hour. Daily exchange in the recirculating seawater system averaged 10%. A 12 h light:12 h dark photoperiod was maintained throughout growth trials. Stocking densities were 35 per m^2 (12 per tank) in the first two experiments, and 114 per m^2 (7 per tank) in the third experiment.

In tank trials, shrimp were fed in excess four times daily at 6 hour intervals (06:00, 12:00, 18:00, and 24:00) to make intact feed pellets continuously available to shrimp and at the same time minimize the amount of excess feed left in tanks. The amount fed each day was divided into four equal feedings at 6 hour intervals. Uneaten food was partially removed by the flow of water through the tanks as the feed pellets disintegrated. Residual feed pellets, exuviae, and dead shrimp were manually removed on a daily basis by siphoning. Although the amount of food presented daily to each shrimp did not differ between dietary treatments, feed rates (as percentages of the weights of the shrimp) varied between dietary treatments because of differences in growth between treatments. Daily feed rates in the three tank experiments ranged from 20 to 41, 20 to 25, and 18 to 74%, respectively.

The pen growth trial was conducted in 1 m^2 square pens at a seawater depth of 0.76 m. Pens were constructed of 4 x 15.9 mm polyethylene mesh, Aquanet^R XV-1110, InterNet^C Incorporated. Daily exchange of sea water in the pond was 2%. Shrimp were stocked at a density of 60 m^{-2} and fed at a daily rate of 3% of their weight as estimated from maximum growth rates obtained under similar conditions

in previous growth trials. The amount fed per day was divided equally among four separate feedings at 6 hour intervals (06:00, 12:00, 18:00, and 24:00). Although the amount of feed presented to shrimp did not differ between dietary treatments, feed rates increased up to a level of 4% during the growth trial due to a lower than expected growth rate.

In tank trials, salinity, temperature, and dissolved oxygen were measured daily and ammonia, nitrite, and nitrate were measured weekly. In the pond trial, salinity, maximum and minimum temperatures, and morning and afternoon dissolved oxygen were measured daily. Salinity was controlled by the addition of fresh water. Average weights of shrimp were determined at the beginning and end of growth trials by dividing the total weight of shrimp in the tank by the number of shrimp. Growth of shrimp was expressed in terms of instantaneous growth rates (IGR) given by the following equation.

$$IGR = 100 \times (\ln(Weight_{final}/Weight_{initial}))/28 \text{ days}$$

Formaldehyde residues in muscle tissue were determined by the laboratory of Dr. Chavez at the McDonald Campus of McGill University, Ste Anne de Bellevue, Quebec, Canada and provided for this manuscript by BASF Aktiengesellschaft (Kohler, 1986).

One-way analysis of variance (ANOVA) and Student-Newman-Kuels *a posteriori* comparison of means were used to determine significant differences in survival and growth due to levels of polymethylolcarbamide in the feed. Differences in concentration of formaldehyde residues in shrimp tissues were analyzed by linear regression of tissue levels onto levels of polymethylolcarbamide in the feed. The critical probability value used to determine significance was $P = 0.05$.

RESULTS

Water quality parameters are given in Table 2 for the tank experiments and in Table 3 for the pen experiment. Water quality parameters were stable within experiments and adequate for growth and survival of shrimp. Within experiments,

Table 2. Water quality parameters for tank experiments. Values represent mean ± standard deviation for number of replicates in parentheses.

Parameter	Experiment 1	Experiment 2	Experiment 3
Salinity (ppt)	31 ± 1 (29)	24 ± 1 (29)	29 ± 1 (28)
Temperature (°C)	28 ± 1 (29)	28 ± 1 (29)	29 ± 1 (28)
Oxygen (ppm)	6.0 ± 0.4 (29)	6.0 ± 0.2 (29)	7.7 ± 0.6 (28)
Ammonia (ppm N)	0.11 ± 0.08 (3)	0.06 ± 0.02 (4)	0.04 ± 0.01 (4)
Nitrite (ppm N)	0.04 ± 0.02 (3)	0.21 ± 0.08 (4)	0.03 ± 0.03 (4)
Nitrate (ppm N)	0.25 ± 0.25 (3)	1.19 ± 0.15 (4)	not determined

survival did not differ among shrimp fed different levels of polymethylolcarbamide ($P = 0.3315$ in tank experiment 1, $P = 0.2466$ in tank experiment 2, $P = 0.9807$ in tank experiment 3, and $P = 0.5222$ in the pen experiment). Means $\pm$ standard deviations (number of replicates) for percentages of survival were 95 $\pm$ 6 (40), 88 $\pm$ 8 (40), 66 $\pm$ 11 (25), and 57 $\pm$ (17) in the respective experiments.

Table 3. Water quality parameters for pen experiment. Values represent mean $\pm$ standard deviation for number of replicates in parentheses.

Salinity (ppt)	35 $\pm$ 2 (28)
Daily maximum temperature (°C)	29 $\pm$ 2 (28)
Daily minimum temperature (°C)	25 $\pm$ 2 (28)
Morning dissolved oxygen (ppm)	6 $\pm$ 1 (28)
Afternoon dissolved oxygen (ppm)	9 $\pm$ 2 (27)

Growth of shrimp fed diets containing polymethylolcarbamide are shown in Figure 1. The first tank experiment indicated that dietary polymethylolcarbamide levels of 1% or above reduced growth of shrimp. In addition, growth of shrimp fed a diet containing 8% polymethylolcarbamide was significantly lower than that of shrimp fed diets containing 1, 2 and 4% polymethylolcarbamide. Differences in growth between shrimp fed diets containing 1, 2, and 4% polymethylolcarbamide were not significant. In the second tank experiment, dietary polymethylolcarbamide levels similar to those used in commercial feeds reduced growth. Instantaneous growth rates of shrimp fed 0.5 and 1% polymethylolcarbamide were significantly lower than those of shrimp fed feed without polymethylolcarbamide. In the third tank experiment, 1% polymethylolcarbamide feed also reduced growth. Instantaneous growth rates of shrimp fed a feed bound with 2% alginate were greater than those of shrimp fed a feed bound with 1% polymethylolcarbamide under conditions similar to commercial applications. In contrast to results obtained in the second indoor tank experiment, growth of shrimp fed a diet containing 0.5% polymethylolcarbamide in outdoor pens did not differ from that of shrimp fed a diet without polymethylolcarbamide.

In tank experiment 1, mean weight gains per shrimp were reduced 58% by the 8% polymethylolcarbamide feed relative to the weight gain (4.1 g) of shrimp fed the feed without polymethylolcarbamide. Mean weight gains were reduced 19 to 26% by feeds containing 1, 2 and 4% polymethylolcarbamide. In tank experiment 2, the mean weight gain (2.36 g) of shrimp fed feed without polymethylolcarbamide was reduced 29 and 30% by feeds containing 0.5 and 1% polymethylolcarbamide, respectively. In tank experiment 3, the mean weight gain (1.71 g) of shrimp fed feed without polymethylolcarbamide was reduced 30% by feed containing 1% polymethylolcarbamide.

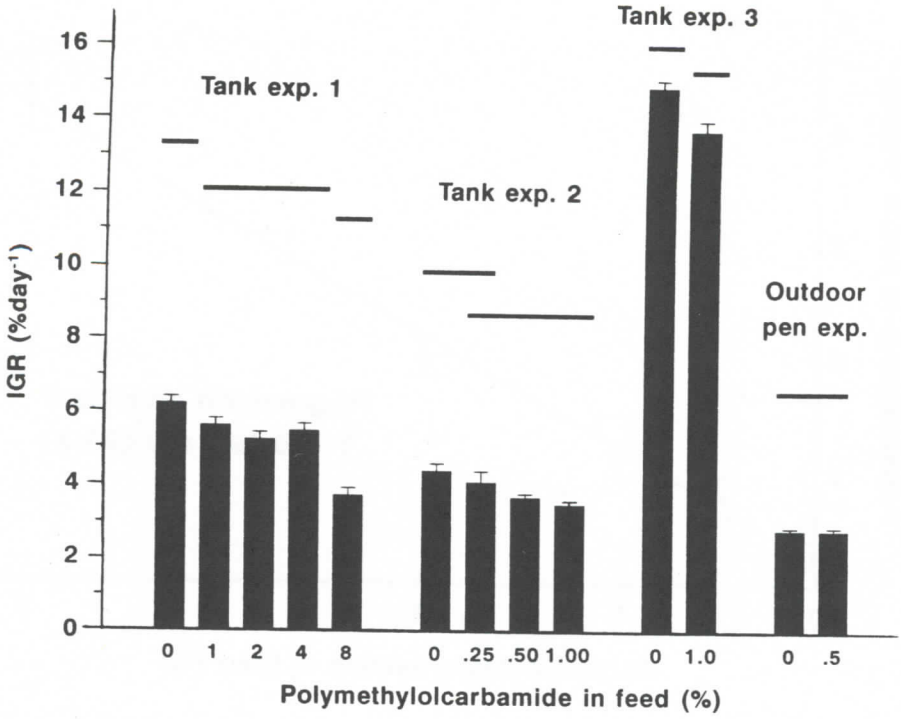

Figure 1. Effect of polymethylolcarbamide on instantaneous growth rate (IGR) of *Penaeus vannamei* in percent growth per day. Natural foods were available to shrimp in the outdoor pen experiment but not in tank experiments 1, 2, and 3. Vertical bars represent means of 8 replicates except for 0% polymethylolcarbamide in the pen experiment where 9 replicates were used and tank experiment 3 where 7 replicates were used. Error bars represent standard errors of means. Means under a continuous horizontal line are not significantly different.

At the end of the first tank experiment, muscle tissue of shrimp was collected for analysis to determine if formaldehyde residues accumulated from the polymethylolcarbamide in the feed (Figure 2). Regression of tissue formaldehyde residues onto polymethylolcarbamide levels in the feed indicated that tissue formaldehyde was linearly proportional to polymethylolcarbamide in the feed and described by the regression equation shown in Figure 2. Deviations from linear regression were not significant ($P = 0.7549$). To test the significance of linear regression of tissue formaldehyde onto polymethylolcarbamide in the feed, the mean square for linear regression was tested over the pooled mean squares for deviations from linear regression and for variation within feed levels according to rules proposed by Bancroft (1964). Linear regression was highly significant ($P < 0.0001$).

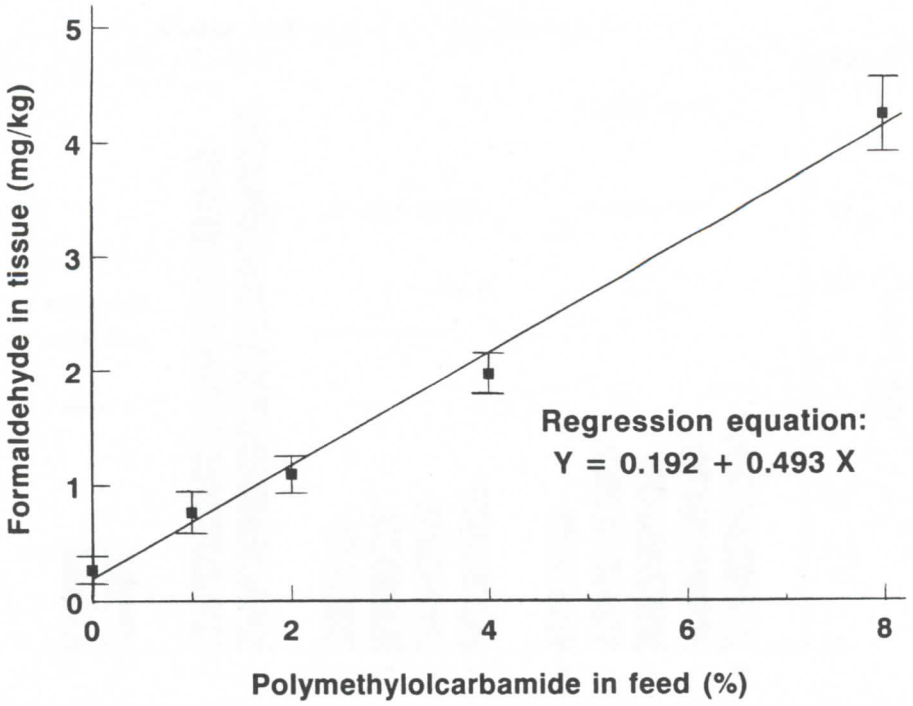

Figure 2. Effect of polymethylolcarbamide in feed on residual formaldehyde in muscle tissue of *Penaeus vannamei* on a dry weight basis. Data are means of 8 replicates and error bars represent standard errors of means.

DISCUSSION

Data presented in this study indicate that polymethylolcarbamide levels at or above 0.5% can reduce growth of shrimp. Reductions in growth may occur at levels below 0.5% but were beyond the resolution of the experimental design.

Instantaneous growth rates were lowest in the pen experiment and highest in the third tank experiment. The major reasons for differences in growth rates between experiments were differences in the size of shrimp used. Means $\pm$ standard deviations (number of replicates) for initial weights were 2.38 $\pm$ 0.42 g (100) in the pen experiment, and 0.89 $\pm$ 0.12 g (40) and 0.93 $\pm$ 0.11 g (40) in the first two indoor tank experiments. In the third tank experiment, the mean initial weight was estimated from a random sample of shrimp at stocking as 0.026 g. Other factors that may have contributed to lower growth in the outdoor pen experiment were higher stocking densities and lower seawater temperatures at night. Diurnal variations in water temperature did not occur in the indoor experiments.

Data presented in this study for the pen experiment indicate that under conditions where shrimp have access to natural foods, growth was not reduced by the presence

of 0.5% polymethylolcarbamide. However, feeding rates and feed quality were not equivalent in the tank and pen experiments because feeding rates were higher in the tanks, and because formulated feeds may be supplemented with natural foods in outdoor pens. Notwithstanding these differences, growth in the pen experiment was not limited by the availability or quality of formulated feeds. In preliminary experiments conducted to establish optimal feeding rates for similar feeds in the pen experimental system, higher daily feeding rates ranging from 5 to 10% did not increase growth of shrimp stocked at the same density and biomass. Stable isotope studies conducted in similarly constructed pens by Anderson et al. (1987) indicated that pond productivity contributed 53 to 77% of the carbon in growth of shrimp with a final biomass of 86 to 96 g m^{-2}. Although final biomass in the present pen study was greater (177 to 182 g m^{-2}) than that reported by Anderson et al. (1987), natural foods were also important in the present study. In the pen experiment, the most probable explanation for the lack of reduction in growth by the feed containing 0.5% polymethylolcarbamide is that availability of natural foods reduced dietary intake of polymethylolcarbamide relative to the indoor tank experiments even where the percentage of polymethylolcarbamide in the formulated feeds was the same.

Recommended practical application rates for use of polymethylolcarbamide as a binder in feeds for terrestrial animals are 0.1 to 0.3% (BASF, 1983b; Uniscope, n. d.). Levels reported to be suitable and effective for pellet binding in shrimp feed are 0.4 to 0.5% (Boonyaratpalin, 1984) and 0.5 to 0.75% (Uniscope, 1989). There was no indication from the results of this study that polymethylolcarbamide reduced growth when used at recommended levels for semiintensive pond culture. However, as culture methods are intensified and amounts of pelleted feed consumed by shrimp increase relative to the availability of natural foods, growth may be decreased by polymethylolcarbamide levels that have no effect in less intensive culture. Under these conditions, economic advantages of using polymethylolcarbamide as a pellet binder would have to be weighed against its negative effect on growth.

Concerns about formaldehyde residues in tissues of shrimp grown on feeds bound with polymethylolcarbamide are unwarranted. Low levels of formaldehyde are present in many foods, including shrimp. In the first tank experiment, muscle tissues of shrimp fed feed without polymethylolcarbamide contained a mean formaldehyde concentration of 0.27 mg kg^{-1} on a dry weight basis, or 0.06 mg kg^{-1} on a wet weight basis (dry weight = 23.9% of wet weight). Formaldehyde is also formed in shrimp and fish from the decomposition of trimethylamine oxide to dimethylamine and formaldehyde as the result of deterioration during processing or storage (Amano and Yamada, 1964; Castell and Smith, 1973). At 1 to 4°C, formaldehyde levels on a wet weight basis in defrosted muscle of fish were 20 mg kg^{-1} in *Gadus macrocephalus* after 2 days of storage, and 50 mg kg^{-1} in *Hexagrammos stelleri* after 4 days of storage (Amano and Yamada, 1964). For old frozen fillets stored under poor conditions, Castell and Smith (1973) reported a formaldehyde concentration of 0.85 mmole per 100 g of muscle, a level equivalent to 255 mg kg^{-1} on a wet weight basis. In shrimp, postmortem storage for 72 hours at 7°C and one year at -20°C produced formaldehyde residues on a wet weight basis of 1.2 and 1 mg kg^{-1}, respectively (Hose and Lightner, 1980). Using the regression equation in Fig. 2, predicted muscle formaldehyde residues in shrimp that were fed diets containing 0.5 % polymethylolcarbamide in tanks would be 0.44 mg kg^{-1} on a dry weight basis or 0.10 mg kg^{-1} on a wet weight basis. At levels of

polymethylolcarbamide recommended for pellet binding, levels of formaldehyde in shrimp tissues would be lower than those reported in stored fish and shrimp.

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