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An Evaluation of Five Types of Binders To Improve the Artificial Diet Of Young American Eels

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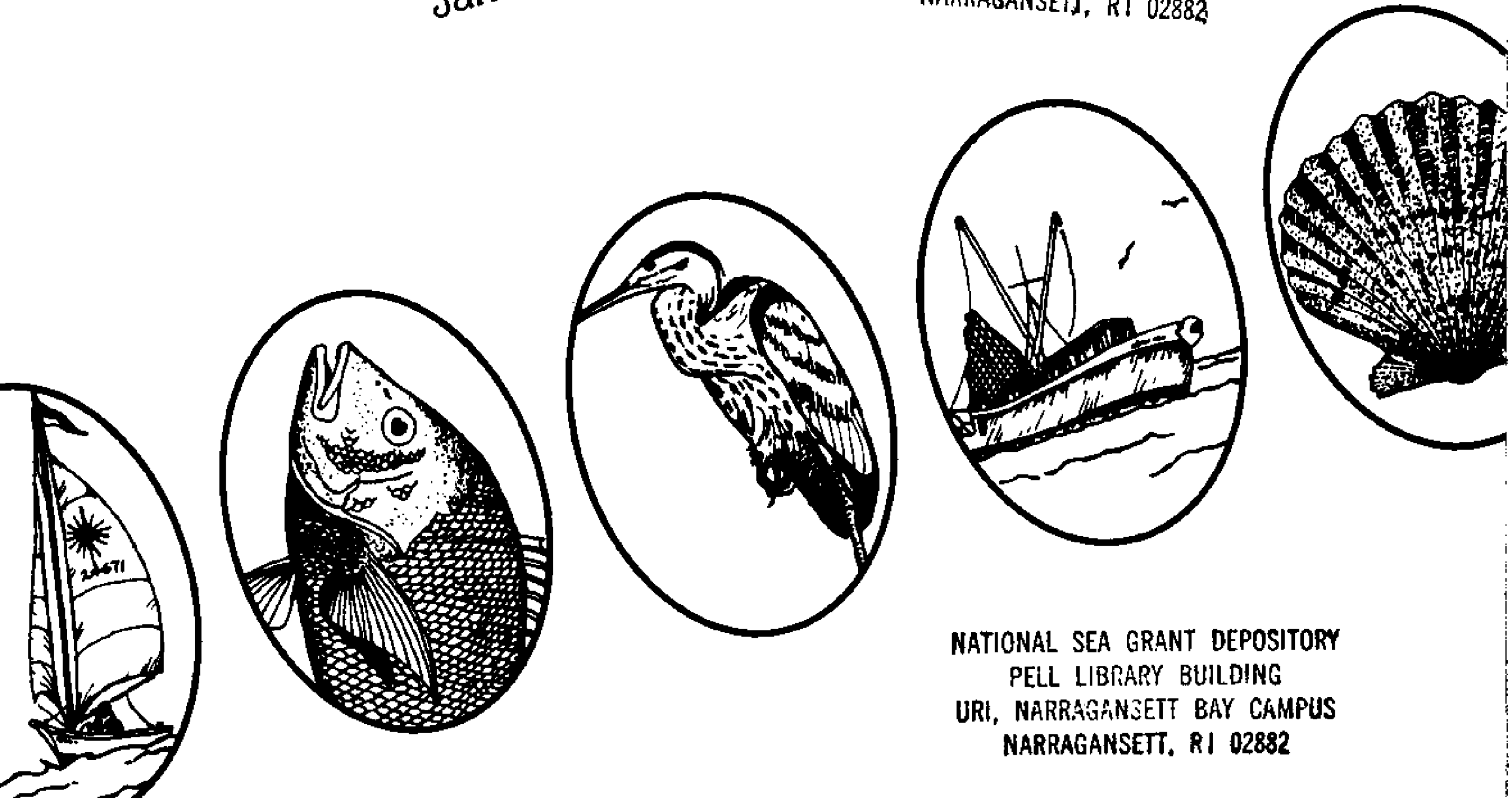

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AN EVALUATION OF FIVE TYPES OF BINDERS

TO IMPROVE THE ARTIFICIAL DIET

OF YOUNG AMERICAN EELS

(*Anguilla rostrata* Le Seuer)

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ABSTRACT

The "traditional" diet of young American eels (*Anguilla rostrata*) consists of fish meal, alpha cornstarch and a vitamin premix. This formulation has been used at the University of North Carolina Sea Grant Aquaculture Demonstration Project in Aurora, North Carolina, and in commercial eel farming operations in Japan and Taiwan. The main problems with this mixture are decreased profit margins due to wasted food and a general lowering of water quality.

The percentages of each of the binders completely evaluated were as follows: (1) 1 and 2 percent Kelco Gel LV (a low viscosity alginate), (2) 1, 2, and 4 percent CMG (carboxymethylcellulose), (3) 1 and 2 percent GFS (xanthan-locustbeam-guar gum), (4) 1 percent wheat gluten and (5) the standard formulation. These mixtures were then presented to young eels to evaluate food acceptance, crude protein leaching rate and the amount of particulates suspended during feeding periods of 10, 20, and 30 minutes.

All of the diets were accepted by the eels. Crude protein leaching rates were highest in the standard formulation and the diet containing wheat gluten. The lowest rates were found in 1 and 2 percent GFS, 4 percent CMC and 1 percent Kelco Gel LV which were not significantly different. One percent wheat gluten and the standard formulation permitted the highest particulate fallout from the sample, while 1 and 2 percent CMC, 1 percent Kelco Gel LV and 2 percent GFS (not significantly different) permitted the least.

From this evaluation it was determined that 1, 2, and 4 percent CMC, 2 percent GFS and 1 percent Kelco Gel LV performed better than the other formul-

ations. Suggested formulations contain different percentages of CMC as it was the most cost effective ($3.00 per 0.454 kg).

INTRODUCTION

Anguillid eels are a commercially important species in many countries such as Japan, Taiwan, Italy, Spain, Germany, France and the United States (Liewes 1978). The only country with a long history of eel culture is Japan where the Japanese eel, _Anguilla japonica_, has been farmed commercially for about 150 years and on a subsistence basis for centuries longer (Bardach, Ryther and McLarney 1972). After World War II, Taiwan became the second country where this species was cultured. The culture of _Anguilla rostrata_ in the United States is in its early stages. A great deal of developmental work is being done in the U.S., especially at the University of North Carolina Sea Grant Aquaculture Demonstration Project in Aurora, North Carolina. The main objectives of the work at the facility are to modify existing technology and methodology for use with the American eel, and to make eel culture a more economically feasible endeavor in the United States.

A diet consisting of 75 percent fish meal and 25 percent alpha cornstarch, supplemented with a vitamin premix and an antibiotic, has been used at the Aurora facility (Angel and Jones 1974). This formulation was based on work done in Japan (Arai et al 1972; 1974; Nose and Arai 1972). The main problems with this diet are a general lowering of water quality and decreased profit margins due to wasted food. If the food, to be presented to the elvers, is not bound tightly enough it will break down very quickly and foul the water. This may lower levels of the dissolved oxygen and increase ammonia which is critical in an intensive culture situation. Fouling can also lead to an invasion of the system by pathogens. Either of these factors or a combination of both may lead to high mortality or a highly stressful situation in which slower growth occurs.

The objective of this study was to evaluate five types of binder (Table 1) in order to improve the artificial diet for young American eels.

MATERIALS AND METHODS

A preliminary study was conducted to determine the amount of time necessary for the standard formulation, consisting of fish meal, alpha cornstarch, a vitamin premix and water, to stabilize (absorb its maximim amount of water). This was accomplished by immersing 2.5 g samples of the mixture in 2 liters of water. The samples were removed at approximately four-minute intervals and placed on an Instron Universal Testing Machine (Bourne 1978). Hardness (amount of force required to compress the sample to 1.4 mm) and cohesiveness (amount of force required to pull the sample apart) were then determined for a two-hour period.

Different percentages of each binder were evaluated in order to provide a range for selection. The criterion for selection of the percentages was similarity to the standard formulation in terms of hardness, cohesiveness and tactile observations. The binders were added to the standard formulation by replacing an equal amount of alpha cornstarch with an equal amount of binder.

The diets containing carboxymethylcellulose (CMC), wheat gluten and alpha cornstarch were thoroughly mixed before and after water was added. Special preparation was necessary for the Kelco Gel LV and GFS formulations. The Kelco Gel LV diet was first dry mixed, then, after water was added, placed in

Table 1. Listing of binders used in this evaluation and where they were obtained.

Binder	Obtained From
Alpha cornstarch	McKesson Chemical Co. Charlotte, N. C.
Carboxymethylcellulose (CMC)	Hercules, Inc. Wilmington, Delaware
Alginate (Kelco Gel LV)*	Kelco, Inc. San Diego, California
Xanthan-Locust bean-Guar gum (GFS)	Kelco, Inc. San Diego, California
Wheat gluten	Industrial Grain Products Montreal, Quebec

* LV - Low Viscosity

a two percent calcium bath to enhance its binding properties. The GFS was placed in water at 24°C and then added to the other components of the formulation.

Samples from each mixture were placed in two liters of water and removed at two-minute intervals for the first 10 minutes and 10-minute intervals thereafter, up to 60 minutes. Upon removal, the samples were placed on the Instron Universal Testing Machine to determine hardness and cohesiveness. These measurements were compared to the standard, and selection of specific percentages for further evaluation was made.

Five eels, measuring approximately 8.5 by 0.3 cm, were placed in each of 27, wide-mouth jars containing 2.84 liters of water, two hours before feeding to allow them to become adjusted to their new environment. The jars were then divided into nine sets, one set for each formulation. Each jar in a set was labeled 10, 20, or 30 minutes (the amount of feeding time), respectively.

To present the formulations, feeding baskets were constructed of 3.175 mm wire-mesh screening. The shape of the baskets was similar to those used by Rickards, Foster and Jones (1978). The baskets were lowered to approximately one-third the total depth in the jar immediately preceding introduction of the eels. Prior to feeding, the eels were starved for approximately 48 hours to insure complete gut evacuation. A 2.5 g sample was placed into each jar and feeding was then allowed for that time period which was marked on the jar (10, 20 or 30 minutes).

After each time interval the baskets containing excess food (not eaten) were removed. The excess food was taken out of each basket, placed in pre-weighed aluminum foil strips and transferred to a drying oven. A temperature of 104 ± 2°C was maintained for five hours to insure drying (anonymous 1969). Each was then weighed on a Mettler P1000 balance.

The eels were removed from the jars and the remaining water was vigorously stirred to create a homogenous mixture of particles (dissolved and undissolved). To determine the amount of crude protein leached from the samples, four 50-ml samples from each mixture were placed in 100-ml Whirlpac plastic bags and frozen. Total nitrogen was determined by the standard Kjeldahl method and micro-grams protein were calculated as 6.25 times micro-grams nitrogen.

To determine the amount of undissolved particulates, the remaining water was filtered through a dried pre-weighed 11.0 cm micropore filter. Filters were then placed on aluminum foil dishes and placed in a drying oven. A temperature of 104 ± 2°C was maintained over a five-hour period to insure complete drying (Anonymous 1969). After drying, each was then weighed on a Mettler P1000 balance.

RESULTS

From the preliminary study it was determined that the standard formulation stabilized at approximately 90 minutes (Figure 1). There was a decrease in hardness and cohesiveness from 4.8 g to 2.2 kg and from 1.8 kg to 1.2 kg respectively. In the analysis of variance (Table 2) the time parameter accounted for most of the variation in both models and consequently was highly significant. R-squares were 0.89 and 0.81 respectively, implying a good fit for both models.

Using the data from the Instron Universal Testing Machine it was determined that eight percent and four percent of each binder, except CMC, were found either to be too hard or too cohesive. CMC was an exception in that four percent was within the range necessary to meet the criterion for the

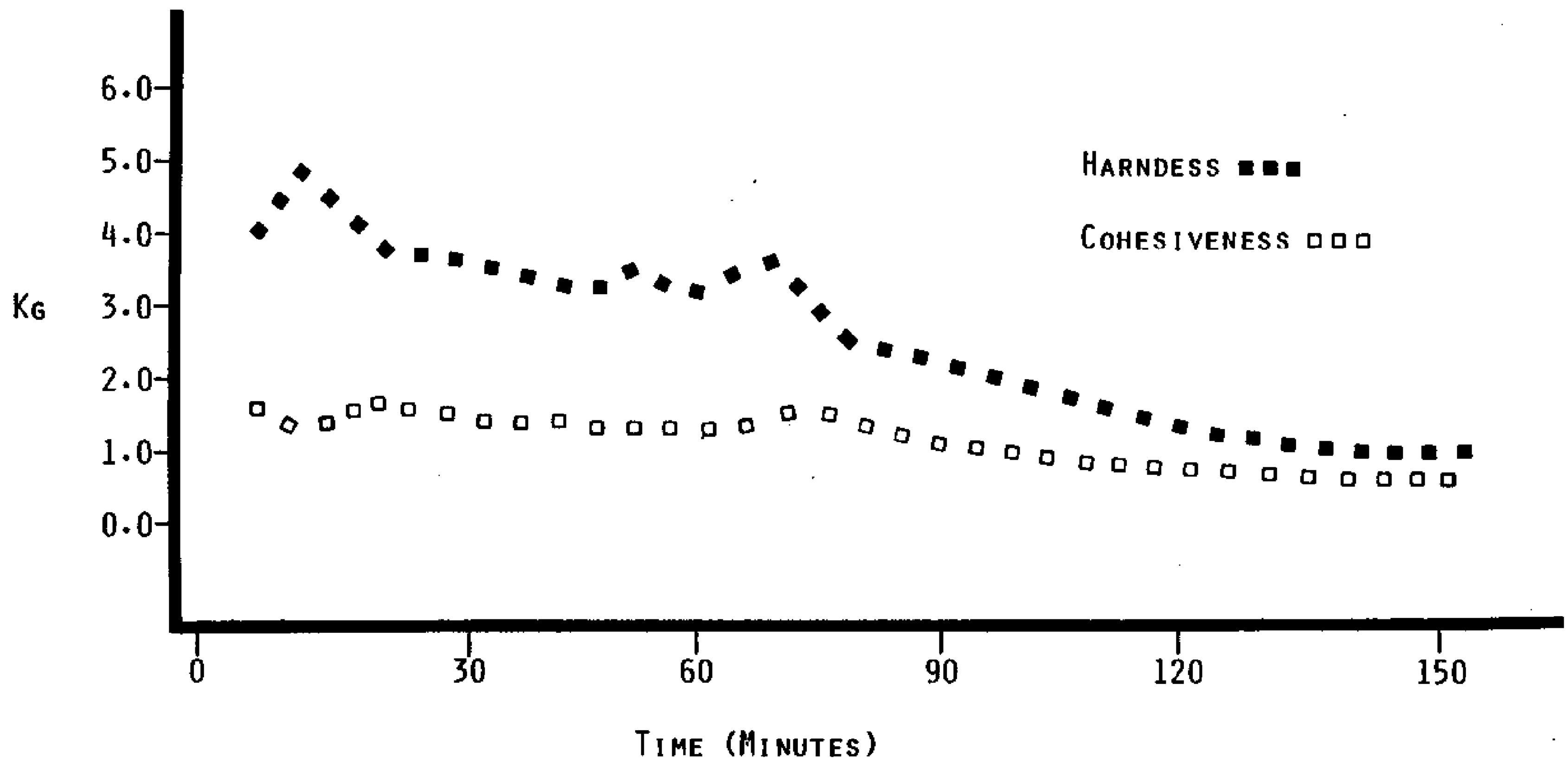

FIGURE 1. RELATIONSHIP BETWEEN HARDNESS AND COHESIVENESS OVER TIME FOR THE STANDARD FORMULATION

Table 2. F-values for hardness and cohesiveness of the standard formulation.

Source	D. F.	Standard Formulation Hardness F-values	Standard Formulation Cohesiveness F-values
Time	1	387.91 ***	169.94 ***
Block	1	1.71 NS	0.03 NS
Interaction of time by block	1	0.24 NS	1.46 NS
Error	50		

NS - Not Significant.

*** - Highly Significant (.0001).

selection. In the case of wheat gluten, the only percentage completely evaluated was one percent. It appeared that the higher percentages of wheat gluten inversely affected the binding properties of the alpha cornstarch (lowered hardness and cohesiveness). This was also indicated by visual observations which showed a more rapid breakdown at higher percentages. A list of binders and those percentages which were used throughout the remainder of the experimentation is given in Table 3.

The mean values of the amount of excess food remaining in the baskets are given in (Table 4). One percent alginate had the highest mean (1.68 g) while wheat gluten had the lowest (0.76 g). In the analysis of variance (Table 5), the variables time and the interaction of binder and time were significant, while the variable binder was highly significant and accounted for the largest portion of the variation. The R-square value was 0.91 indicating a good fit of the model.

Crude protein leached to a greater degree (Table 3) with wheat gluten and the standard formulation while it was least for 1% Kelco Gel LV, 1% GFS, 2% GFS and 4% CMC (not significantly different). All variables in the analysis of variance (Table 5) were highly significant, implying that they all contributed to the variation. The R-square for the model was 0.86 thus indicating a good fit of the independent variables and the dependent variable crude protein.

The wheat gluten diet released the greatest amount of particles into the water (1.14), while 1% CMC, 2% CMC, 1% GFS and 2% GFS (not significantly different) released the least (Table 4). All variables in the analysis of variance (Table 5) were highly significant. This indicated, as in the crude protein analysis, that they all contributed to the variation in the proced-

Table 3. Mean values of dry weights for excess food (g), crude protein (ug) and filtrand (g)

Formulation	Excess Food (g)	Crude Protein (ug)	Filtrand (g)
Standard Formulation	0.85	0.007	0.96
1% Kelco Gel LV	1.68	0.003	0.11
2% Kelco Gel LV	1.21	0.005	0.26
1% CMC	1.41	0.005	0.08
2% CMC	1.49	0.004	0.09
4% CMC	1.54	0.004	0.18
1% GFS	1.57	0.004	0.23
2% GFS	1.66	0.003	0.09
1% Wheat Gluten	0.76	0.009	1.14

Table 4. Formulative quantities for the diets evaluated, their individual costs and the total cost per pound of diet.

	Fish Meal		Alpha Cornstarch		Vitamin Pre-mix		Binder		0.454 kg Total Cost Per Pound ($)
	Amount (g)	Cost ($)	Amount (g)	Cost ($)	Amount (g)	Cost ($)	Amount (g)	Cost ($)	
Standard Formulation	340.5	0.18	113.5	0.14	9.00	0.29			0.61
1% Kelco Gel LV	340.5	0.18	108.96	0.13	9.00	0.29	4.54	0.04	0.64
2% Kelco Gel LV	340.5	0.18	104.42	0.13	9.00	0.29	9.08	0.08	0.68
1% CMC	340.5	0.18	108.96	0.13	9.00	0.29	4.54	0.03	0.63
2% CMC	340.5	0.18	104.42	0.13	9.00	0.29	9.08	0.06	0.66
4% CMC	340.5	0.18	95.34	0.12	9.00	0.29	18.16	0.09	0.68
1% GFS	340.5	0.18	108.96	0.13	9.00	0.29	4.54	0.03	0.63
2% GFS	340.5	0.18	104.42	0.13	9.00	0.29	9.08	0.06	0.66
1% Wheat gluten	340.5	0.18	108.96	0.13	9.00	0.29	4.54	0.01	0.61

Table 5. F-values for excess food, crude protein and the filtrand.

Source	D. F.	Excess Food Dry Weight F-values	Crude Protein F-values	Filtrand Dry Weight F-values
Binder	8	32.20 ***	76.16 ***	16.29 ***
Run (Binder)	18	1.67 NS	24.27 ***	4.99 ***
Time	2	4.89 *	27.94 ***	87.55 ***
Binder (by Time)	16	3.17 *	12.39 ***	3.31 **
Error	36			

NS - Not Significant.

* - Significant (.01).

** - Very Significant (.001).

*** - Highly Significant (.0001).

ure. The R-square value was 0.98, indicating a close relationship between the independent variables and the dependent variable, filtrand. I was determined that the percentage of food wasted (filtrand) was not correlated with the amount of food eaten but was correlated with time.

DISCUSSION

The selection of binders to be evaluated presented a major problem in this study. As each binder will bind differently under different circumstances (Tyre C. Lanier,[1] personal communication), binding properties were not used as a means of selection. (The criterion for the selection of the binders was that each has been previously used in the aquaculture industry.) All binders selected have been used in diets fed to various aquatic animals with the exception of Kelco Gel LV (Arai, Nose and Hashimoto 1972; Angel and Jones 1974; Kalin 1979; and Passanza 1978). Meyers, Butler and Hastings (1980) used, Kelgin HV, an alginate in their work with larval bullfrog diets. Kelco Gel LV was selected instead of the Kelgin HV because of its relative ease to work with and cost when compared to Kelgin HV (Alan King,[2] personal communication).

From the preliminary study it was determined that after 90 minutes the standard formulation had stabilized and was ready to be presented to the eels. This fact was of prime importance in obtaining reproducible results. Water stability was also important from an economic standpoint. Kalin (1979) states "the water stability of the diet contributes to the feeding efficiency, reduces pollution and disease, and helps minimize drain on oxygen."

[1] Food Science Department, N. C. State University, Raleigh, N.C. 27650.
[2] Kelco Technical Service Laboratory, Clark, N.J. 07066.

The mixtures chosen for further evaluation were similar in hardness and cohesiveness to the standard formulation which was found to be an adequate diet at the University of North Carolina Sea Grant Aquaculture Demonstration Project in Aurora, N. C. (W. L. Rickards,[3] personal communication).

Degrees of hardness and cohesiveness were essential to the selection of percentages to be evaluated further. Most of the eel's teeth do not protrude very far out of the epidermis (Tesch 1977). If the diet was too hard/or cohesive, the young eels would not have the ability to tear or bite off portions of feed which would in turn reduce the feeding efficiency of the animals.

As mentioned earlier, water stability contributed to the feeding efficiency. It was for this reason that the only percentage of wheat gluten to be completely evaluated was one percent. Higher percentages seemed to hinder the binding qualities of the alpha cornstarch, thus lowering the values of hardness and cohesiveness. In diets to determine nutrient leaching from pelleted rations, Goldblatt, Conklin and Brown (1979) used 15 percent wheat gluten in one of their test formulations (except the standard) which suggests wheat gluten and the standard formulation are inadequate in terms of their binding capabilities.

The only binder to be evaluated at the four percent level was CMC. At this level the hardness and cohesiveness were close to the lower percentages of CMC. This suggests that at these percentages there was not a significant increase in the hardness and/or cohesiveness beyond criterion limits. Arai, Nose and Kawatsu (1974) and Yone (1975) used CMC at the ten percent level. It alone was used as the binding agent. In this study it was shown that if CMC

[3] University of North Carolina Sea Grant Program, N.C. State University Raleigh, N. C. 27650.

was used to enhance the binding qualities of the alpha cornstarch, much lower percentages could be used to obtain results similar in hardness and cohesiveness to the control.

Meyers, et al. (1980) found that GFS achieved good water stability at the three percent level. Here again, as with Arai's work, GFS was the only binding agent in the diet. This study has shown that if GFS was used in conjunction with alpha cornstarch, lower percentages could be used.

The amount of excess food in each of the baskets was used to determine acceptance of the diet by the eels. All were accepted suggesting that the binders did not add foul odors or flavors. Feeding variation and sloughing of food from the sample were important to the amount of excess food, therefore one could not state that the animals preferred one diet over another.

The amount of crude protein leaching was shown to vary between binders. Wheat gluten and the standard formulation containing only alpha cornstarch allowed the most leaching to occur. This suggests their inadequacy in terms of maintaining the protein level in the diet. Those that permitted the least amount of leaching, 1% GFS, 4%f CMC, 1% Kelco Gel LV and 2% GFS, were therefore considered to be adequate binders. Goldblatt, Conklin and Brown (1979) found little significant loss of protein due to leaching in their test formulations bound with an alginate.

The filtrand (amount of food wasted) was also used to aid in the evaluation. Wheat gluten and the standard formulation containing only alpha cornstarch allowed the most particulates into the water column, again suggesting their inadequacy as binding agents. It should be noted again, that by itself, alpha cornstarch was thought to be adequate until these different binders were added. Those that allowed the least amount of particulates into the water, 1% Kelco Gel LV, 2% CMC, 2% GFS and 1% CMC, were therefore considered to be satisfactory. As the percentage of food wasted was not correlated with the amount eaten, it was suggested that the amount of particulates and the amount of crude protein did not solely rely on the feeding activity but also on the natural breakdown of the diet.

The cost of each of the diets was analyzed as the final step in the evaluation. The diets containing CMC were the most cost effective. Since CMC diets performed well in all aspects of this study, it is suggested for use in the diets of young American eels.

The standard formulation, while comparable in cost, proved to be inadequate in terms of maintaining crude protein levels and the release of particulates into the water column. It should be noted that Ke'co Gel LV was one of the most expensive alginates. Other less expensive alginates, with some modifications, could function in eel diets.

From this evaluation it was determined that the suggested formulations should contain either one, two or four percent CMC. The reason for this range of percentages is that as the eels grow, their jaw musculature becomes stronger thus permitting them to tear off larger portions of food and creating the possibility of excessive waste. This warrants the need for formulations which hold together longer to help prevent wastage due to feeding activity and natural breakdown. Thus, I have suggested formulations which will make the food available to the animals for a longer period of time. Recommended for a one-pound (454 g) mixture are as follows:

1. 1% CMC formulation: 340.5 g fish meal
   109.0 g alpha cornstarch
   4.54 g CMC
   9.00 g vitamin premix

2. 2% CMC formulation: 340.5 g fish meal
   104.0 g alpha cornstarch
   9.09 g CMC
   9.00 g vitamin premix

3. 4% CMC formulation 340.5 g fish meal
   95.34 g alpha cornstarch
   18.16 g CMC
   9.00 g vitamin premix

Each of the above mixtures should be thoroughly dry mixed. Enough water should be added until a doughy consistency is reached (for a one-pound mixture add approximately 0.908 liters) and then thoroughly mixed again.

From this study it should be clear that while CMC has been suggested for use in eel diets, the other binders tested could be used in other aquatic animal feeds. Further studies with the recommended percentages are necessary to determine any differences in terms of growth and feeding efficiency, overall condition of the animals and water quality.

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