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Effect of Diet Processing Method and Ingredient Substitution on Feed Characteristics and Survival of Larval Walleye, *Sander vitreus*

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Abstract

Two methods were developed for the production of larval fish diets. The first method, micro-extrusion marumerization (MEM), has been tested in laboratory feeding trials for many years and produces particles that are palatable and water stable. The second method, particle-assisted rotational agglomeration (PARA), produced diets that have lower density than diets produced by MEM. Each method was used to produce diets in the 250- to 400- and 400- to 700-μm range and compared with a reference diet (Fry Feed Kyowa* [FFK]) for feeding larval walleye in two experiments. The effect of substituting 4% of the fish meal with freeze-dried artemia fines was also investigated. In the first experiment, 30-d survival was greater ($P < 0.05$) for fish fed a diet produced by PARA without *Artemia* (49.1.0%) than for fish fed the same diet produced by MEM (27.6%). The addition of *Artemia* to a diet produced by MEM did not increase survival of larval walleye. Fish fed the reference diet had 24.4% survival. In the second experiment, there was an effect of both processing method and *Artemia* supplementation, and an interaction of these effects, on survival. Fish fed a diet produced by PARA without *Artemia* supplementation had 48.4% survival, and fish fed the same diet produced by MEM had only 19.6% survival. Inclusion of 4% freeze-dried *Artemia* improved ($P < 0.04$) survival of fish fed MEM particles but not those fed PARA particles. Fish fed FFK had greater weight gain than fish fed other diets in both experiments. Data indicate that the PARA method of diet processing produces smaller, lower density particles than the MEM process and that diets produced by the PARA process support higher survival of larval walleye with low capital and operating costs.

Production of walleye fingerlings and other small-egged species has traditionally relied upon pond rearing or feeding live *Artemia* during the first 30 d of culture (Colesante et al. 1986). The development of specialized larval diets (e.g., BioKyowa) and modified rearing techniques (Loadman et al. 1989; Barrows et al. 1993) now allow for intensive larval walleye culture using only formulated diets. In the late 1980s, survival greater than 10% was considered good for larval walleye fed formulated diets (Barrows et al. 1988; Loadman et al. 1989). With the rearing techniques and diets now available, survival rates as high as 50% are observed in a production hatchery (A. Moore, Iowa Department of Natural Resources, Moravia, IA, USA, personal communication), although tank production of larval walleye is very limited. Walleye production is primarily for sport-fishing purposes, with only a few attempts being made at food fish production. Modern commercial larval diets differ from traditional steam-pelleted crumbles in both composition and method of manufacture, and they possess greater water stability and palatability than traditional fry diets. However, the precise method of manufacture and ingredient composition of commercial larval diets remains proprietary.

The U.S. Fish and Wildlife Service had been engaged in development of open-formula larval diets for use in fisheries restoration programs (Barrows 1990, 1991, 1994, 1995). Numerous methods of particle formation were tested during

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this development program, but two techniques that borrowed equipment from the pharmaceutical industry demonstrated the greatest potential. This equipment, which includes a radial discharge extruder and a marumerizer, imparts the desired physical characteristics to the particles and exposes the diet to comparatively low temperatures. The first method, microextrusion marumerization (MEM), is effective in producing particles as small as 500 μm in diameter (Barrows and Lellis 1996). Diet ingredients are extruded through a 500-μm screen then fractured, shaped, and densified in the marumerizer. The second method, particle-assisted rotational agglomeration (PARA), produces diet without the extruder by rotating a wet mash in the marumerizer with inert beads. The PARA method is particularly useful when a higher yield of particles less than 500 μm are required, such as for culture of species like the walleye, striped bass, or other small-egged fish. The objective of this study was to determine the physical characteristics of diets produced by the two processing methods and to compare their effects on growth and survival of larval walleye with that of a commercial larval diet. Improved diet formulations and manufacturing technologies are anticipated to increase the efficiency of larval fish production and eliminate dependence upon live diets and pond rearing.

Materials and Methods

Fish and Experimental Design

Two feeding trials were conducted using 275-L cylindrical larval rearing tanks supplied with recirculating water (Barrows et al. 1993). Rearing temperature was initially 16 C increasing to 21 C over 5 d and maintained throughout each trial. Tanks were illuminated 24 h each day with overhead fluorescent lights and cleaned daily by siphoning and pressure spraying the center screen when necessary. Dissolved oxygen and ammonia levels were monitored biweekly and determined to be adequate throughout both trials.

Each experiment used five thousand five hundred 1-d-old walleye fry counted with a Jensorter Fry Counter (Kindschi and Barrows 1991) per tank. Feeding began at 3-d posthatch. Belt feeders dispensed a measured quantity of diet uniformly throughout each 24-h period. Diet particles ranging from 250 to 400 μm were fed during the first 7 d, followed by 400- to 700-μm particles for the remainder of the trials. Feeding rate was based on a hatchery constant of 72 (metric), a predicted growth rate of 0.12 cm/d, and survival of 100 and 75% for Weeks 1 and 2, respectively, and 60% thereafter.

Four experimental and one commercial diet were used in the trials. A base walleye starter diet was formulated from krill meal, egg solid, and herring meal as the major protein-containing ingredients (Table 1). This diet was modified by substituting 4% freeze-dried *Artemia* for 4% herring meal, and both formulations were manufactured using the MEM and PARA methods. The commercial diet was Fry Feed Kyowa (FFK;

TABLE 1. *Ingredient composition (g/kg) of experimental walleye starter diets.*

Ingredient	0% *Artemia*	4% *Artemia*
Krill meal[a]	380	380
Egg solid[b]	200	200
Herring meal[a]	214	174
Liver meal[c]	70	70
Artemia meal[d]	0	40
Yeast extract[e]	20	20
Menhaden oil[f]	40	40
Binder (TIC 515)[g]	30	30
Vitamin premix[h]	30	30
Ascorbic acid[e]	10	10
Trace mineral premix[i]	5	5
Inositol[e]	1	1

[a] International Proteins Corp., New York, New York, USA.

[b] International Ingredient Corp., St. Louis, Missouri, USA.

[c] American Protein Corp., Ames, Iowa, USA.

[d] INVE Aquaculture, Grantsville, Utah, USA.

[e] Amersham Life Sciences, Arlington Heights, Illinois, USA.

[f] Zapata Haynie Corp., Hammond, Louisiana, USA.

[g] TIC Gums, Inc., Belcamp, Maryland, USA.

[h] Contributed per kilogram of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D_3, 720 IU; vitamin E (as DI-α-tocopheryl-acetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulfate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B_{12}, 30 mg.

[i] Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 1.

BioKyowa, Inc., Chesterfield, MO, USA) and was added to the experiments as a reference diet. This diet series was chosen as a reference diet because it is used by the hatchery producing the most larval walleyes with formulated feed (Rathbun State Fish Hatchery, Rathbun, IA, USA). FFK-B-400 was fed for 1 wk, followed by FFK-C-700 for the remainder of each trial (Barrows et al. 1993). Experiment 1 involved three experimental diets (MEM 0%, MEM 4%, PARA 0%) and FFK, each fed to three tanks of larval walleye for 30 d. Experiment 2 had four experimental diets and FFK, each fed to four tanks of fish. The second experiment was terminated after 23 d because of mechanical problems with the recirculating system. At the end of each trial, all remaining fish were hand counted and group weighed. Performance was evaluated in terms of survival and growth.

Diet Preparation

The MEM process involved two pieces of equipment for the production of particles. An LCI, Inc. (Charlotte, NC, USA) system consisting of a radial discharge EXDC(F)S-60 extruder and a QJ-400 marumerizer was used. All diet ingredients were combined and mixed in a Marion paddle mixer prior to addition of fish oil. Water was then added to the mix (32%, weight to weight) and then extruded through a 500-μm screen. The mash was extruded at an auger speed of 19 revolutions per minute (rpm) to form wet noodles. The noodles were then placed in the marumerizer, which consists of a cylindrical chamber with a rotating bottom plate. The plate is grooved to impart energy from the machine to the diet. This energy breaks the noodles, reshapes, and densifies the particles. The marumerizer is equipped with a variable speed motor to allow for a range of 300–1210 rpm. The noodles were first processed for 10 sec at 1060 rpm followed by approximately 90 sec at 500 rpm. The shaped particles were then placed in an ambient temperature (~18 °C) forced-air dryer until moisture levels were less than 10% as determined with 30 min cycles of 125 C on an OHaus MB 200 Moisture Analyzer (OHaus Corp., Florham Park, NJ, USA). The diet was then sifted to size and stored in nitrogen-flushed, vacuum-packed plastic bags at room temperature. All diets were fed within 120 d. Using the 500-μm screen, the MEM process resulted in approximately 3% yield of particles between 250 and 400 μm and 97% between 400 and 700 μm. Yield of 250- to 400-μm-size particles can be increased to nearly 8% by adjusting moisture content of the mash and marumerizer speed.

The PARA method (U.S. Patent no. 5,851,574) was developed primarily to increase the yield of particles less than 400 μm relative to the MEM method (Barrows and Rust 2000). This method differs from traditional spheronization because only a cylindrical shaper, a marumerizer (LCI, Inc., Charlotte, NC, USA), is used and the process does not involve an extruder (Barrows and Hardy 2001). Yields of 35–45% of 250- to 400-μm-size particles are routinely obtained with the PARA method, but diet formulation and moisture content of the mash will affect yields. A quantity of wet mash (~35% added moisture) is placed directly into the marumerizer with a charge of 3.0-mm inert particles (75%, weight of beads/weight of wet mash). The rotation of the marumerizer imparts energy to the inert particles, which in turn transfers energy to the mash, producing spheroid particles in a wide distribution of sizes. Contact time in the marumerizer was optimized to 45 sec for all diets. The mash and inert beads are discharged from the marumerizer and sifted through a 2.0-mm screen to remove the beads. The diets were then air-dried at ambient temperatures, sifted to appropriate sizes, and stored similarly to the MEM diets. This is a low-pressure agglomeration method relative to MEM particles (Barrows and Lellis 2000).

Analyses

Proximate analyses of diets were conducted on triplicate samples. Protein was determined by microkjeldahl with a Tecator Kejeltec Auto Analyzer (model 1030 Tecator, Höganäs, Sweden). Lipid was determined by ether extraction, moisture by drying at 95 C for 24 h and weighing, and ash by exposure of 0.5 g samples to 600 C for 2 h and weighing (AOAC 1990).

Diet particles in the 400- to 700-μm range were evaluated for density, sink rate, and water stability. Density was determined by placing

a 20 g sample in a 20-mL graduated cylinder and recording the occupied volume. This method results in lower values than water displacement method because of air between individual particles but was used because of potential differences in water absorption among the different diets. Sink rate was determined as the average rate of descent of 30 individual particles over a 30-cm distance within a 1000-mL graduated cylinder filled with water at room temperature (~18 C). Water stability measured the combined effects of leaching and particle degradation. Six 5 g samples of each diet were placed in 20-mL vials of water and inverted five times. After 5 min, three vials of each diet were poured through a Pall Type A/E glass fiber filter (1.0-μm nominal openings) (Pall Life Sciences, East Hills, NY) and the retained material dried overnight at 90 C. The dry material was then sifted for 3 min through 700- and 400-μm screens using a Rotap-sifter (W.S. Tyler, Inc., Gastonia, NC, USA). The purpose of the 700-μm screen was to break apart particles that had adhered to one another. All material retained between the 400- and 700-μm screen were considered individual particles and not reagglomerations. The weight of particles greater than 400 μm was expressed as a percentage of initial weight for the water stability score. This same procedure was repeated with the remaining three vials after 24 h.

Dietary characteristics and fish performance data were analyzed with the general linear models procedure of the Statistical Analysis System (SAS Institute 1988). Any value expressed as a percentage was arcsine transformed prior to analysis (Sokal and Rohlf 1981). Differences in treatment means for Experiment 1 were separated using the Waller/Duncan multiple range test.

Results

Physical Characteristics

The experimental diets were analyzed to contain 526–556 g/kg crude protein and FFK 603 g/kg crude protein (Table 2). Because of the high levels of krill meal in the experimental diets, and the high level of nonprotein nitrogen associated with the exoskeletons of crustaceans, the level of digestible protein in the experimental diets was assumed to be lower than the crude protein values would indicate. The lipid level of FFK was slightly higher than the experimental diets, and the moisture content much less. Ash content was uniform among all diets.

TABLE 2. *Nutrient composition (g/kg) of diets on an as-fed basis.*

Diet	Protein	Lipid	Ash	Moisture
MEM, 0% *Artemia*	535	158	93	82
MEM, 4% *Artemia*	556	168	94	73
PARA, 0% *Artemia*	546	155	93	78
PARA, 4% *Artemia*	526	147	91	98
Fry Feed Kyowa	603	179	90	28

MEM = microextrusion marumerization; PARA = particle-assisted rotational agglomeration.

The density of the experimental diets varied according to processing method but not diet formulation (Table 3). Diets produced by MEM had an average density of 0.72 g/cc. The PARA process can be adjusted to produce particles of different densities, but the previously described methods produced diets with an average density of 0.67 g/cc. FFK-B-400 had a lower density (0.5 g/cc) than the experimental diets. The density of the diet corresponded directly to the observed rate of sinking of the particles. The MEM diets had a mean rate of sinking of 1.65 cm/sec as compared to 1.22 cm/sec for the diets produced by the PARA process and 1.03 cm/sec for the FFK-B-400 diet.

The apparent water stability (AWS) of diets produced by MEM was higher ($P < 0.01$) than that of PARA-produced diets (Table 3). The 5-min AWS for MEM diets averaged 75.9% compared to 65.2% for the PARA particles. After 24 h in water, the AWS values decreased to 69.8 and 64.2% for MEM and PARA particles, respectively. The majority loss of water-soluble nutrients and particulate matter occurred for both particle types within the first 5 min of water exposure. Averaging across all diets, a total loss of 29% occurred during initial 5 min compared to only 4% additional loss during the next 23 h and 55 min. These data suggest that the diets possess a particle matrix that quickly leached fine particulate matter but generally remained structurally intact over time. Addition of *Artemia* to the diet significantly increased stability among

TABLE 3. *Mean ± SEM of physical characteristics of diets.*

Diet	Density (g/cc)	Sink rate (cm/sec)	Stability, 5 min (%)	Stability, 24 h (%)
MEM, 0% *Artemia*	0.72 ± 0.04	1.66 ± 0.04	72.6 ± 0.2	67.5 ± 0.5
MEM, 4% *Artemia*	0.72 ± 0.03	1.64 ± 0.04	79.3 ± 0.1	72.2 ± 0.4
PARA, 0% *Artemia*	0.67 ± 0.03	1.11 ± 0.04	67.4 ± 0.3	64.7 ± 0.7
PARA, 4% *Artemia*	0.67 ± 0.01	1.33 ± 0.12	63.0 ± 0.4	63.7 ± 0.6
FFK	0.50 ± 0.02	1.03 ± 0.15	Reagglomerated	Reagglomerated
ANOVA, P[a]				
Processing method	0.03	0.01	0.01	0.01
Artemia	0.83	0.26	0.03	0.06
Method × *Artemia*	0.67	0.17	0.02	0.01

MEM = microextrusion marumerization; PARA = particle-assisted rotational agglomeration.

[a] Fry Feed Kyowa (FFK) is not included in statistical analysis.

MEM particles but significantly decreased stability among PARA particles, creating a significant interaction between processing method and *Artemia* supplementation. The BioKyowa diet reagglomerated upon drying, so determination of stability by using the described method was not possible.

Feeding Trials

In the first experiment (Table 4), survival of fish consuming diet produced by PARA without *Artemia* (49.1%) was equal to fish fed the MEM-produced diet with *Artemia* (39.9%) but was greater than fish fed the MEM-produced diet without *Artemia* (27.6%). There was no effect of diet processing method or *Artemia* supplementation on weight gain. Fish fed the reference diet had a relatively low survival (24.4%) but were much heavier at trial end (1048 mg/fish) than fish fed the experimental diets (average 308 mg/fish).

In the second experiment (Table 5), survival was affected by both processing method and *Artemia* supplementation. Fish fed diets processed by MEM averaged 29.6% survival compared to 46.4% for fish fed diets processed by PARA ($P < 0.01$). *Artemia* supplementation affected survival ($P < 0.04$), and there was an interaction of processing method and *Artemia* supplementation ($P < 0.05$). Addition of *Artemia* to the MEM diet increased survival from 19.6 to 39.5% ($P < 0.03$), but addition of *Artemia* to the PARA diet had little effect (48.4% no *Artemia*, and 44.4% with *Artemia*). Similar to the first experiment, fish fed the BioKyowa diet had much greater weight gain than fish consuming experimental diets, and there were no differences among experimental diets.

TABLE 4. *Mean ± SEM of 30-d survival and weight of larval walleye fed diet produced by MEM and PARA in Experiment 1.*

Diet	Survival (%)	Individual wet weight (mg)
MEM, 0% *Artemia*	27.6 ± 9.9[b]	360 ± 223[b]
MEM, 4% *Artemia*	39.9 ± 9.5[ab]	249 ± 62[b]
PARA, 0% *Artemia*	49.1 ± 5.4[a]	316 ± 77[b]
FFK[1]	24.4 ± 4.4	1048 ± 345

MEM = microextrusion marumerization; PARA = particle-assisted rotational agglomeration. Means within a column followed by different superscript letters are significantly different ($P < 0.05$).

[1] Fry Feed Kyowa (FFK) is not included in statistical analysis.

Discussion

The higher pressure used in the extrusion phase of the MEM process resulted in a denser, faster

TABLE 5. *Mean ± SEM of survival and weight of larval walleye fed diets produced by MEM and PARA in Experiment 2.*

Diet	Survival	Individual Wet Weight (%) (mg)
MEM, 0% *Artemia*	19.6 ± 8.6	111 ± 6
MEM, 4% *Artemia*	39.5 ± 5.2	111 ± 29
PARA, 0% *Artemia*	48.4 ± 9.4	120 ± 8
PARA, 4% *Artemia*	44.4 ± 4.4	90 ± 9
FFK	46.5 ± 3.8	223 ± 41
ANOVA, P[a]		
Processing method	0.01	0.69
Artemia	0.04	0.08
Method × *Artemia*	0.05	0.08

MEM = microextrusion marumerization; PARA = particle-assisted rotational agglomeration.

[a] Fry Feed Kyowa (FFK) is not included in statistical analysis.

sinking particle than particles made by the PARA process. This higher density may be the basis for the greater AWS of the MEM particles relative to PARA particles. The AWS values account for both soluble and particulate matter loss. Although these values are probably not comparable to stability values derived by other methods, the described technique does provide a relative assessment of binding strength within the pellet. Differences in AWS can be attributed to manufacturing technique because the formulations and added moisture levels were identical for the two processing methods.

The 5-min AWS values did not differ greatly from the 24-h values among the experimental diets. This observation suggests that the particles readily leached water-soluble components upon initial submersion, whereas the main binding systems remained stable over time. Both these characteristics could be advantageous if the diets were produced to contain enough water-soluble nutrients for metabolic needs at the moment of consumption, after compensating for some initial leakage. Leaching of specific nutrients is believed to be necessary for diet acceptance by the fish. Incorporation of water-stable microcapsules into the PARA particles may provide the best combination of nutrient delivery and diet acceptance. The carrier particles could either completely retain water-soluble nutrients and be digestible or leach nutrients at a predetermined and controlled rate. The use of this type of complex particle has been effective in diets for penaeid shrimp (Villamar and Langdon 1993).

Survival was greater for fish fed PARA particles without *Artemia* than for fish fed MEM particles without *Artemia* in both feeding trials. This increase in survival may have resulted from increased diet acceptance because of softer texture or increased digestibility of the less dense PARA particles. Additionally, the slower rate of sinking of PARA particles may have made them more available to fish, thereby increasing diet consumption and survival.

Weight gain for fish fed the experimental diets was much less than for fish fed the reference diet in both trials. The majority of loss in matter occurred in the experimental diets when tested for AWS that occurred in the first 5 min. It is assumed that both fine particulate matter and water-soluble nutrients were lost from the experimental diets. The slow growth of fish fed the experimental feeds, compared to fish fed the reference diet, may be because of the rapid leaching of water-soluble nutrients or less feed consumption because of the faster rate of sinking. The reference diet reagglomerated when processed to determine water stability, so a comparison was not possible. Methods to improve retention of water-soluble nutrients in PARA or MEM particles may increase weight gain and should be investigated.

The PARA process offers several advantages over the MEM process for the production of larval fish diets. The production of high yields of low-density particles less than 400 μm and superior fish performance have been noted earlier and are important. Equally important are the practical aspects of diet production on a commercial scale. The need for only one piece of processing equipment reduces both initial investment and operating costs. Production of particles greater than 1.0 mm using the PARA process, however, is difficult to control. Additionally, once the walleye are large enough to consume a 1.0-mm particle, they feed aggressively enough that a slower rate of sinking is not necessary. From both a feed production and fish performance standpoint, the MEM or another extrusion method for particles greater than 1.0 mm is desirable.

This paper reports an open-formula diet that can support survival equivalent to commercially marketed larval diets. Nutrient requirement trials with larval fish are rare because of an inability to easily package the nutrients in a form that the fish will consume. Also, production of successful experimental diets in the laboratory has been inadequate. The development of the PARA process and practical type formulations provide the tools necessary to further develop larval fish diets and to begin nutrient requirement determinations for larval fish.

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