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Evaluation of Acid-insoluble Ash as an Indicator for Digestibility Determination with Red Drum, *Sciaenops ocellatus*, and Hybrid Striped Bass, *Morone chrysops* × *M. saxatilis*

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Red drum, *Sciaenops ocellatus*, and hybrid striped bass, *Morone chrysops* × *M. saxatilis*, are important fish species for warmwater aquaculture worldwide, and their culture continues to increase because of their popularity in regional and international markets. The rapid development of the industry requires further refinement of dietary formulations to allow more efficient use of feedstuffs as well as to limit waste excretion. Apparent digestibility determinations of various feed ingredients are therefore very important to develop cost-effective diet formulations, evaluate ingredient quality, and limit the excretion of nutrients into the environment, which may cause environmental problems (Gatlin and Hardy 2002; Montaño-Vargas et al. 2002).

The traditionally used marker in digestibility determinations with both red drum and hybrid striped bass has been chromic oxide (Cr_2O_3) (Gaylord and Gatlin 1996; McGoogan and Reigh 1996; Rawles and Gatlin 2000; Li et al. 2004b). However, its use is restricted to formulated diets, and it requires rather complicated analytical procedures. Furthermore, some studies with various fish species have reported that it passes through the digestive tract at a rate different from that of food (Bowen 1978; Kaushik and Oliva-Teles 1985) and can influence nutrient digestibility, especially that of carbohydrates (Shiau and Liang 1995). Shiau and Lin (1993) also reported that small amounts of chromic oxide can be absorbed into tissues, which can cause underestimation of digestibility. Acid-insoluble ash (AIA), either from feedstuffs which contain high levels of AIA, such as fish meal or meat and bone meal, or from supplementation of Celite®, a form of diatomaceous silica, has been reported to be an effective internal or external marker in digestibility studies with humans (Rowan et al. 1991), chicken (Cheng and Coon 1990), and some fish species including rainbow trout, *Oncorhynchus mykiss* (Atkinson et al. 1984); Arctic charr, *Salvelinus alpinus* (Gurure et al. 1996); channel catfish, *Ictalurus punctatus* (Shahat 1993); and tilapia, *Oreochromis aureus* (Goddard and McLean 2001). Because of its low expense and ease of analysis, AIA is a desirable alternative for determination of digestibility in fish. However, AIA is not a perfect marker as some studies with different fish species (Tacon and Rodrigues 1984; Wetherbee and Gruber 1993; Perera et al. 1995; Morales et al. 1999) have reported limited utilization of AIA. Because the use of AIA as an inert marker would facilitate digestibility evaluations of commercial diets and ingredients, the present study was designed to determine the suitability of AIA as an indicator of digestibility studies in red drum and hybrid striped bass.

Materials and Methods

Experimental Diets and Preparation

Two experimental diets were used for the red drum trials. These diets used menhaden fish meal as the sole protein source and were formulated to contain 40% crude protein and 8.2%

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lipid with an estimated digestible energy value of 3.5 kcal/g (Table 1). These diets satisfied and/or exceeded all known nutrient requirements of red drum (Gatlin 2002). Either chromic oxide (Fisher Scientific International Inc., Hampton, NH, USA) or Celite (World Mineral Inc., Lompoc, CA, USA) was included in the diet at 1% of dry weight, respectively, to serve as the inert marker. The diets used for the hybrid striped bass trial were based on an experimental feed mix (Kent SeaTech Corp., San Diego, CA, USA) formulated to contain 53% crude protein and 15% lipid with an estimated digestible energy value of 4.0 kcal/g. The three experimental diets for hybrid striped bass were prepared from the basal mixture and supplemented with either 1 or 1.5% Celite or 1% Celite plus 0.5% chromic oxide (Table 2). The nutritional profile of this diet exceeded all the known requirements of hybrid striped bass (Webster 2002). All diets were pressure pelleted through a 5-mm die using a meat grinder and then air-dried at 25 C and stored at −20 C until fed (Webb and Gatlin 2003).

TABLE 1. *Dietary components and analyzed composition (dry weight basis) of diets in the red drum experiment.*

	1% Chromic oxide	1% Celite
Ingredient		
Menhaden fish meal[a]	60	60
Dextrin[b]	20	20
Menhaden oil[c]	2.5	2.5
Mineral premix[d]	4.0	4.0
Vitamin premix[d]	3.0	3.0
Carboxymethyl cellulose[b]	4.0	4.0
Cellulose[b]	5.5	5.5
Celite[e]	1.0	0
Chromic oxide[f]	0	1.0
Analyzed composition		
Protein (%)	39.5	41.7
Lipid (%)	9.3	8.6
Ash (%)	17.7	17.5
Chromic oxide (%)	1.02	0
Acid-insoluble ash (%)	1.96	2.98

[a] Special Select (Omega Protein, Inc.). Contained 67.8% protein and 10.7% lipid on a dry weight basis.
[b] US Biochemical Corp. (Cleveland, OH, USA).
[c] Omega Protein, Inc. (Reedville, VA, USA).
[d] Same as Li et al. (2004a).
[e] World Mineral Inc.
[f] Fisher Scientific International Inc.

TABLE 2. *Dietary components and analyzed composition (dry weight basis) of diets in the hybrid striped bass experiment.*

	1% Celite	1.5% Celite	1% Celite/0.5% Chromic oxide
Ingredient			
Fish meal[a]	36.1	36.0	36.0
Soybean meal[a]	17.1	17.0	17.0
Feather meal[a]	8.4	8.4	8.4
Flour[a]	12.8	12.7	12.7
Blood meal[a]	12.4	12.3	12.3
Menhaden oil[a]	15.0	15.0	15.0
Vitamin premix[a]	0.6	0.6	0.6
Choline chloride	0.3	0.3	0.3
Stay C[a]	0.2	0.2	0.2
Calcium propionate[a]	0.1	0.1	0.1
Trace mineral[a]	0.2	0.2	0.2
Celite[b]	1.0	1.5	1.0
Chromic oxide[c]	0	0	0.5
Analyzed composition			
Protein (%)	51.0	51.4	51.4
Lipid (%)	18.3	18.3	18.8
Ash (%)	7.3	7.8	8.0
Chromic oxide (%)	0	0	0.3
AIA (%)	1.3	1.7	1.1

[a] Nelson's Silver Cap Fish Feeds.
[b] World Mineral Inc.
[c] Fisher Scientific International Inc.

Chemical Analysis

The diets and fecal samples were analyzed in the Fish Nutrition Laboratory at Texas A&M University. Dry matter was determined by placing samples in an oven at 135 C for 2 h after which ash values were determined by combusting the samples at 550 C for 3 h (AOAC 1990). Crude protein was determined on a Labconco Rapid Still II distillation apparatus as Kjeldahl nitrogen (AOAC 1990) and calculated as percent nitrogen multiplied by 6.25. Chromium oxide was determined spectrophotometrically (Furukawa and Tsukahara 1966) for the red drum trials and by atomic absorption spectrometry for the hybrid striped bass trial. AIA was analyzed following the procedure described by Atkinson et al. (1984). Recovery of AIA was determined to be higher than 95%.

Red Drum Culture and Fecal Collection

In the first trial, juvenile red drum, *S. ocellatus* (average 3.8 g/fish), were originally obtained from Texas Parks and Wildlife Department

(TPWD, Corpus Christi, TX, USA) and raised at the Texas A&M University Aquacultural Research and Teaching Facility (ARTF) for 8 mo. After this period, the fish (120 ± 4.7 g/fish, mean ± SE) were stocked (18 fish/tank) into a recirculating system consisting of six 1200-L circular fiberglass tanks. The system was operated as a closed recirculating system with a common corrugated-plate settling chamber and a common biological filter (Rawles and Gatlin 2000). Water flow rate was maintained at approximately 5–7 L/min. Salinity was maintained at 8 ppt using a commercial seawater mixture (Fritz Chemical Co., Dallas, TX, USA), stock salt, and well water. Water temperature was controlled by conditioning the ambient air in the building and remained at 26 ± 1 C. Water quality (dissolved oxygen, ammonia, and nitrite) was measured weekly to ensure optimum environmental conditions. The chromium and Celite diets were randomly assigned to three tanks of fish each. Fish were fed to apparent satiation twice daily. Feeding began 4 d prior to the first fecal collection. Fecal samples were collected 4 h postfeeding by the stripping technique (Austreng 1978; Gaylord and Gatlin 1996) every third day for 21 d, pooled per tank, dried at 60 C overnight and stored in a refrigerator for analysis.

In the second trial, red drum were obtained from TPWD and subjected to a 2-wk conditioning period in the same culture system as Experiment 1. Each tank was stocked with 35 fish averaging 306 ± 10.4 (mean ± SE) g each. Water quality was maintained as previously described. The chromium and Celite diets were randomly assigned to three tanks of fish each. Fecal samples were collected 5 h postfeeding as previously described.

Hybrid Striped Bass Culture and Fecal Collection

Juvenile hybrid striped bass were originally obtained from Keo Fish Farms (Keo, AR, USA) and raised at the ARTF until they reached a size (~300–500 g) suitable for use in the digestibility trial. Fish were stocked at a density of 30 fish/tank into the same recirculating system used for the red drum trials except that nine tanks were used instead of six. Water temperature was kept at approximately 26 C through ambient heating, and salinity was kept at 3 ppt using a mixture of commercial sea salt (Fritz Chemical Co.), stock salt, and well water. Water flow through the biofilter was kept as high as possible and averaged approximately 7–8 L/min. Each diet (1% Celite, 1.5% Celite, and 1% Celite/0.5% chromium) was randomly assigned to three tanks, and fish were fed to satiation twice daily. Fish were allowed to acclimate to the experimental diets for 1 wk before the first fecal collection. Fecal samples were collected 2.5 h postprandial through the stripping technique. A second fecal collection was performed 2 d after the initial collection, and samples from both collections were pooled for each tank to provide sufficient quantities for analysis.

Digestibility Estimation and Statistical Analysis

The digestibility coefficients (DCs) for organic matter and protein were computed using the following formula: $DC = 100 - (100 \times \%$ indicator in feed/% indicator in feces$) \times (\%$ nutrient in feces/% nutrient in feed). Data from all trials were subjected to ANOVA and Tukey's multiple-range test by SPSS® (SPSS Inc., Chicago, IL, USA). Differences in treatment means were considered significant at $P < 0.05$.

Results and Discussion

In both red drum trials, fish readily accepted the prepared diets and no mortality was observed in either trial. In the first trial, apparent digestibility coefficients (ADCs) of organic matter and crude protein for fish fed the chromic oxide diet were calculated based on both chromic oxide and internal AIA as compared to the ADC calculated based on total AIA for fish fed the Celite diet (Table 3). ADCs calculated based on chromic oxide provided a reasonable estimation of organic matter and crude protein digestibility (~62.2 and 75.6%); however, ADCs calculated based on either internal AIA in the chromic oxide diet or total AIA in the Celite diet failed to provide a proper estimate of nutrient digestibility. The AIA content in fecal samples from red drum fed either chromic oxide diet or Celite diet 4 h postfeeding was similar or even lower than the dietary concentration in contrast to the increased

TABLE 3. *Calculated percent apparent digestibility coefficient (ADC) determined based on chromic oxide and acid-insoluble ash (AIA) as indicators for red drum.*

Experiment/diet	Indicator	Organic matter ADC %[1]	Protein ADC %[1]
Experiment 1			
Chromium diet	Chromic oxide	62.2 ± 1.4a	75.6 ± 1.8a
	Internal AIA	−15.5 ± 2.6b	23.2 ± 3.0b
Celite diet	Celite	−8.3 ± 1.1b	42.1 ± 3.7b
	P^2	0.0001	0.01
Experiment 2			
Chromium diet	Chromic oxide	66.1 ± 1.4a	77.0 ± 1.6a
Celite diet	Acid-insoluble ash	−37.7 ± 2.1b	8.6 ± 1.5b
	P^2	0.0001	0.0001

[1] Values represent means ± SE of three replicate groups (Experiment 1) or duplicate groups (Experiment 2). Values in a column that do not have the same letter are significantly different at $P \leq 0.05$ based on Tukey's multiple-range test (Experiment 1) or Student's *t*-test (Experiment 2).

[2] Significant probability associated with the *F* statistic.

concentrations of chromic oxide in the fecal samples. In the second trial, fecal samples were collected 1 h later than the first trial (Table 3). The ADCs for organic matter and crude protein calculated based on AIA were much lower than those calculated based on chromic oxide, which was very similar to the first trial.

Aggressive feeding was observed with hybrid striped bass throughout the trial. ADCs calculated based on chromic oxide and AIA for fish fed the 1.0% Celite and the 1.0% Celite/0.5% chromic oxide diets were not significantly different (Table 4). ADCs calculated based on AIA for fish fed the 1.5% Celite diet, however, were significantly ($P < 0.05$) lower than the other two diets, indicating that high AIA inclusion (up to 1.5%) affected the digestibility estimation for hybrid striped bass.

In both trials with red drum, AIA did not show promise to be used as an inert indicator for digestibility estimation. However, this is in direct contradiction to the results from the hybrid striped bass trial. Hybrid striped bass and red drum share very similar anatomy of the digestive system except for the number of pyloric caecae. However, considerable differences in nutrient and energy partitioning have been noticed (Burr et al. 2006). Another potential explanation for this difference between species is the relatively high levels of AIA found in the diets used in the red drum trials. Both the chromium- and the Celite-supplemented diets (containing 1.96 and 2.98% AIA, respectively) contained AIA levels substantially above those found in the 1.5% Celite diet (1.7% total AIA) fed to hybrid striped bass. This suggests that even the constituent AIA of the chromic oxide–supplemented diet fed to red drum is present at levels that prevent it from being used as an inert marker for determination of apparent digestibility.

TABLE 4. *Calculated percent apparent digestibility coefficient (ADC) determined based on chromic oxide and acid-insoluble ash (AIA) as indicators for hybrid striped bass.*

Diet	Indicator	Organic matter ADC %[1]	Protein ADC %[1]
1% Celite	AIA	66.1 ± 0.9ab	69.1 ± 1.8ab
1.5% Celite	AIA	60.3 ± 0.6b	63.9 ± 2.0b
1% Celite/0.5% Cr_2O_3	Cr_2O_3	69.6 ± 2.2a	73.7 ± 1.9a
	AIA	70.5 ± 2.6a	73.7 ± 3.1a
	P^2	0.05	0.05

[1] Values represent means ± SE of three replicate groups. Values in a column that do not have the same letter are significantly different at $P \leq 0.05$ based on Tukey's multiple-range test.

[2] Significant probability associated with the *F* statistic.

The mechanism by which too much AIA in the diet might lead to inaccurate measures of digestibility is not clear. It is likely that AIA passed through the gastrointestinal tract of red drum at a differential rate compared to the digesta. Wetherbee and Gruber (1993) provided convincing evidence that AIA did not remain in a constant proportion in fecal matter and passed through the digestive tract of lemon shark, *Negaprion brevirostris*, at differential rates. Increases in dietary AIA concentration may emphasize these changes in proportion although this may not be the only confounding factor. Other nondigestible materials such as chitin or fiber may add bulk to the digesta, resulting in differential passage of soluble and insoluble compounds. This phenomenon may occur in red drum, hybrid striped bass, and other carnivorous fishes, which would explain the results seen in the present study.

Although AIA has been reported as an ideal inert indicator for digestibility and absorption efficiency determination in numerous fish species such as rainbow trout (Atkinson et al. 1984), Arctic charr (Gurure et al. 1996), channel catfish (Shahat 1993), and tilapia (Goddard and McLean 2001), potential use of AIA in estimation of nutrient digestibility of dietary ingredients in aquatic species is contradictory. For example, AIA was reported to be inappropriate for lemon shark, *Negaprion brevirostris* (Wetherbee and Gruber 1993). In rainbow trout, the utility of AIA is also debatable because ADC values based on AIA have been shown to be comparable (Atkinson et al. 1984; Vandenberg and De La Nouè 2001), higher (Morales et al. 1999), and lower (Tacon and Rodrigues 1984; Perera et al. 1995) than values obtained with other markers.

The underlying reason remains elusive, although the contradiction might be attributable to differences in the passage rate of AIA compared to digesta (Wetherbee and Gruber 1993), sources of AIA (Vandenberg and De La Nouè 2001), or digestive physiology of various fishes, as well as differences in culture environment, nutritional profile, or composition of experimental diets. Morales et al. (1999) suggested absorption of AIA by some fishes through an anomalous route. However, our AIA analysis of the rinsed digestive tract of red drum 4–5 h postfeeding of the same diet showed that AIA recovery from the stomach and intestine was negligible (P. Li and D. M. Gatlin, Texas A&M University, unpublished data).

Although the use of chromic oxide may have some disadvantages, it is still a rather reliable indicator for digestibility determination of red drum as demonstrated by Gaylord and Gatlin (1996) and McGoogan and Reigh (1996). Potential use of other alternative digestibility indicators including acid-insoluble organic matters, yttrium dioxide, titanium dioxide, or radioactive compounds for hybrid striped bass or red drum has not been investigated to the best of our knowledge. It remains to be seen if lower levels of AIA in the diet may allow AIA to serve as an inert marker in red drum. Because of the value of an easily analyzed marker such as AIA in digestibility studies, the use of AIA in red drum diets warrants further investigation.

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