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Apparent Digestibility of Gross Nutrients from Feedstuffs in Extruded Feeds for Rainbow Trout, *Oncorhynchus mykiss*

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Abstract.—The current experiment was performed to determine apparent protein and energy digestibility coefficients of feed ingredients by the fecal stripping technique using extruded diets. The ingredients tested included five fish meals, three terrestrial animal by-products, five plant protein concentrates, four plant meals, and seven low-protein plant ingredients. Protein digestibility differed among fish meals and ranged from 90% for fair and average quality menhaden meal to 97% for anchovy meal. Protein apparent digestibility coefficients (ADCs) in plant concentrates ranged from a low of 89% for rice protein concentrate to a high of 100% for wheat gluten meal. Apparent protein digestibility was lower in plant meals than fish meals with a low of 70% for flaxseed meal to a high of 89% for soybean meal. Low-protein plant meals had generally lower protein ADC from 64% for rice bran to 85% for whole wheat. A similar pattern for energy ADCs was observed; ADCs ranged from 106% for anchovy meal to 32% for whole wheat. In the current trial, divergent protein and energy ADC values were obtained most notably in ingredients known to be high in fiber or have very high starch content. The comparability of ingredients/diets processed by cold pelleting or extrusion thus appears questionable at this juncture.

Reducing the reliance of trout production on fish meals is an ongoing effort worldwide, and the utilization of sustainable plant-based ingredients has become a research focus. A crucial stimulant to these efforts has been the dramatic rise in fishmeal prices coupled with often limited availability (FAO Globefish 2007). Researchers around the world are making progress not only in defining the nutrients that are limiting to trout but also in identifying conditionally indispensable nutrients that are present in fish meals but absent from alternate protein sources (Gaylord et al. 2006). Glencross et al. (2007) recently reviewed strategies of evaluations for aquaculture feedstuffs and highlighted five key evaluation components: ingredient characterization, ingredient digestibility measurements, ingredient palatability, determination of nutrient utilization or interferences from an ingredient, and ingredient functionality.

Chemical analyses coupled with ingredient digestibility measurements are often employed as a first step in evaluation of potential aquaculture feedstuffs. These values then serve as the basis for the necessity of further evaluation as described by Glencross et al. (2007). However, currently, most digestibility studies are conducted with cold-pelleted diets and thus may not give accurate estimates of the value of these ingredients in extruded diets. Therefore, the objective of this study was to simultaneously develop a data set of apparent digestibility coefficients (ADCs) in extruded diets for 24 ingredients that are commonly included in aquafeeds or have potential for future use. The ingredients included five fish meals, three animal by-products, five plant protein concentrates, four high-protein (>25% crude protein) plant meals, and seven low-protein (<25% crude protein) plant feedstuffs. Expansion of ingredient evaluation data sets is paramount for the continued sustainability and profitability of the rainbow trout industry and developing these data sets with diet processing techniques that mimic industry methodologies is vital.

Materials and Methods

Ingredients and Diets

The 24 ingredients evaluated were grouped into five classes based on their source and utility

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in aquafeed formulations; fish meals, animal meals, plant protein concentrates, plant protein meals with greater than 25% crude protein, or plant meals of high carbohydrate content and less than 25% crude protein concentration. The methods of Cho et al. (1982) and Bureau et al. (1999) were used to estimate ADCs. Yttrium oxide served as the inert maker. A complete reference diet meeting or exceeding all know nutritional requirements for rainbow trout (NRC 1993) (Table 1) was blended with the test ingredients (Table 2) in a 70:30 ratio (dry weight basis) to form test diets.

All diets were manufactured using a twin-screw cooking extruder (DNDL-44; Buhler AG, Uzwil, Switzerland) with an 18-s exposure to an average of 127 C in the sixth extruder barrel sections. The die plate was water cooled to an average temperature of 60 C. Pressure at the die head varied from 200 to 320 psi, depending on test diet. The 3.0-mm pellets were then dried in a pulse-bed drier (Buhler AG) for 25 min at 102 C with a 10 min cooling period. Final moisture levels were less than 10%. All oil was included in the mix rather than top coated.

Fish and Sample Collection

Rainbow trout, *Oncorhynchus mykiss*, House Creek strain, were obtained from the College of Southern Idaho (Twin Falls, ID) with fifty 250-g fish per 140-L fiberglass tank. Water temperature was maintained at 15 C throughout the feeding trial using flow-through spring water. Lighting was maintained on a 14:10 h diurnal cycle. Each diet was fed to three different tanks of fish over time. Briefly, each diet was randomly assigned to a tank of fish and fed to apparent satiation twice daily for 7 d prior to fecal collection. Fecal samples were obtained in one collection by manual stripping 16–18 h postfeeding. Manual stripping of fish was accomplished by netting and anesthetizing all fish in the tank, followed by gently drying and then applying pressure to the lower abdominal region to express fecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. Thereafter, fish were removed, new fish were stocked, and diets were randomly re-assigned for another 7-d feeding followed by stripping. The process was repeated three times to obtain triplicate fecal samples per feed ingredient for calculation of ADCs. Fecal samples for a given tank were dried overnight at 50 C and stored at −20 C until chemical analyses were performed.

TABLE 1. *Composition of reference diet.*

Ingredient, g/kg (dry weight basis)	
Menhaden fish meal, Special Select™[1]	550
Wheat flour[2]	345
Menhaden fish oil[2]	80
Vitamin C[3]	3
Choline Cl 50%[2]	5
Vitamin premix[2]	6
Trace mineral[2]	1
Yttrium oxide[4]	0.1
Chromic oxide[4]	10
Analyzed composition	
Crude protein, g/kg (dry weight basis)	462.6
Phosphorus, g/kg (dry weight basis)	18.2
Energy, kJ/g	21.34

[1] Omega Protein Corp. (Hammond, LA, USA).
[2] Nelson & Sons Inc. (Murray, UT, USA).
[3] Vitamin C as Rovimix® Stay-C® 35; DSM Nutritional Products (Basel, Switzerland).
[4] Sigma-Aldrich Company (St. Louis, MO, USA).

Chemical Analysis

Dry matter and ash analysis of ingredients, diets, and feces were performed according to standard methods (AOAC 1995). Organic matter was calculated as 100 − ash content. Yttrium and phosphorus were determined in diets and feces by inductively coupled plasma atomic absorption spectrophotometry (University of Idaho Analytical Laboratory Services, Moscow, ID). Crude protein ($N \times 6.25$) was determined in ingredients, diets, and feces by the Dumas method (AOAC 1995) on a Leco TruSpec *N* nitrogen determinator (LECO Corp., St. Joseph, MI, USA). Total energy was determined by adiabatic bomb calorimetry (Parr 1281; Parr Instrument Company Inc., Moline, IL, USA).

ADCs of each nutrient in the test diet and ingredients were calculated according to the following equations (Kleiber 1961; Forster 1999):

TABLE 2. *Analyzed composition of ingredients tested in extruded diets to rainbow trout.*

	Dry matter (%)	Energy (kJ/g)	Crude protein (g/100 g dry weight)	Ash (g/100 g dry weight)	Phosphorus (g/100 g dry weight)
Anchovy fish meal	92.7	22.16	73.1	16.3	2.1
Mexican sardine meal	94.7	21.25	69.5	21.3	3.3
Menhaden fish meal – FAQ	91.5	21.53	73.5	20.3	2.8
Special Select™ menhaden fish meal	93.2	20.35	70.5	19.7	3.0
Sardine fish meal	94.2	23.08	71.8	15.1	2.5
Poultry blood meal, spray dried	89.5	26.10	96.5	1.8	0.08
Feather meal	94.5	26.89	87.4	1.4	0.11
Poultry by-product meal, pet food grade	96.0	23.54	66.4	13.9	2.07
Soy protein concentrate	94.3	21.82	72.2	6.4	0.49
Corn gluten meal	91.0	24.85	70.2	2.1	0.37
Wheat gluten meal	93.4	24.38	83.3	1.0	0.12
Rice protein concentrate 70	92.4	25.41	84.7	1.9	0.22
Barely protein concentrate 25	91.0	21.16	30.5	3.2	0.48
Soybean meal, solvent extracted, dehulled	91.7	20.85	53.9	6.8	0.63
Cotton seed meal	88.6	20.83	46.8	1.1	0.79
Canola meal	91.7	20.98	49.1	6.9	0.86
Flaxseed meal	93.8	18.65	27.5	3.4	0.42
Rice bran	92.4	22.65	17.4	17.9	1.23
Wheat middlings	88.9	19.62	20.7	5.4	0.84
Wheat millrun	88.8	20.26	21.2	5.1	0.73
Barley – Waxbar	91.5	19.62	21.5	2.7	0.28
Wheat, whole	90.3	19.52	16.6	2.0	0.24
Wheat flour	86.9	18.35	13.2	0.5	0.08
Corn, whole	88.6	20.14	10.7	1.7	0.20

$$\text{ADCN}_{\text{diet}} = 100 - 100\{\%\text{Yt in diet} \times \%\text{nutrient in feces}\}/\{\%\text{Yt in feces} \times \%\text{nutrient in diet}\}$$

$$\text{ADCN}_{\text{ingredient}} = \{(a+b)\text{ADCN}_t - (a)\text{ADCN}_r\}b^{-1},$$

where $\text{ADCN}_{\text{ingredient}}$ = apparent digestibility coefficients of the nutrient in the test ingredient, ADCN_t = apparent digestibility coefficients of the nutrient in the test diets, ADCN_r = apparent digestibility coefficients of the nutrient in the reference diet, $a = (1 - p) \times$ nutrient content of the reference diet, $b = p \times$ nutrient content of the test ingredient, and p = proportion of test ingredient in the test diet.

Statistical Analysis

Proc GLM of SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used with Tukey's means separations to ascertain differences between ADCs within each of the five ingredient classes (Tukey 1953). Treatment effects were considered significant at $P < 0.05$.

Results

Fish Meals

There were no differences in energy ADCs for the fish meal products tested, with values ranging from 95 to 106% (Table 3). Protein digestibility was different among the fish meals, with a low of 86% for FAQ menhaden fishmeal and a high of 97% for anchovy fishmeal. Organic matter digestibility was also different among fish meal sources, ranging from 92% for Mexican sardine meal to 105% for anchovy fishmeal.

Animal By-products

Energy digestibility for the animal meals tested ranged from 86 to 88%. The organic matter digestibility for the animal meals ranged from 90

TABLE 3. *ADCs for energy, protein, and organic matter from selected ingredients in extruded feeds for rainbow trout.*[1]

Ingredient	Energy ADC	Protein ADC	OM ADC
Fish meals			
Anchovy fish meal	106	97a	105a
Mexican sardine meal	93	89ab	92b
Menhaden fish meal – FAQ	99	86b	96ba
Special Select™ menhaden fish meal	96	90ab	98ab
Sardine fish meal	95	90ab	95ab
Pooled SEM	2.38	1.64	2.00
Animal by-products			
Poultry blood meal, spray dried	86	91	90
Feather meal	88	87	91
Poultry by-product meal, pet food grade	88	88	92
Pooled SEM	0.53	1.54	1.90
Plant concentrates			
Soy protein concentrate	95ab	99ab	95ab
Corn gluten meal	85bc	92bc	82dc
Wheat gluten meal	99a	100a	98a
Rice protein concentrate 70	90abc	89c	90bc
Barely protein concentrate 25	82c	92bc	80d
Pooled SEM	1.97	1.61	1.20
Plant meals			
Soybean meal, solvent extracted, dehulled	77a	89a	71a
Cotton seed meal	49b	75b	51b
Canola meal	55b	75b	47b
Flaxseed meal	34c	70b	41b
Pooled SEM	2.34	1.33	2.83
Low-protein plant			
Rice bran	60a	64ab	54a
Wheat middlings	36c	68ab	37b
Wheat millrun	37c	69ab	36b
Barley – Waxbar	46abc	57b	37b
Wheat, whole	32c	85a	37b
Wheat flour	56ab	82a	54a
Corn, whole	41bc	66ab	39b
Pooled SEM	3.26	3.32	2.25

ADC = apparent digestibility coefficients, OM = organic matter.

[1] Different lowercase letters within a column for an ingredient class indicate significant differences at $P < 0.05$.

to 92% and protein digestibilities ranged from 88 to 91%. No differences were observed for ADCs among the animal products tested.

Plant Protein Concentrates

The plant protein concentrates were a disparate group of ingredients with protein contents ranging from 30.5 to 84.7%. Apparent energy digestibility coefficients ranged from 82 to 99% within this class of products and ADC of barley protein concentrate (BPC) (82%) was different from that of wheat gluten meal (99%). Energy digestibility of the other products fell within the range of these two ingredients. Differences were also noted between apparent crude protein digestibility for rice protein concentrate (RPC) (89%) as opposed to that of wheat gluten meal (100%). Differences in organic matter digestibility followed the same trend as energy digestibility where the lowest ADC was observed for BPC (80%) and the highest was observed for wheat gluten meal (98%).

Plant Meals

The plant protein meals were widely different for apparent digestible energy. Flaxseed meal had the lowest energy digestibility at 34% and soybean meal had the highest at 77%. Protein digestibility was less variable than energy in these products ranging from 70% for flaxseed meal to 89% for soybean meal, although soybean meal protein digestibility was higher than that of all other ingredients in this class. Similarly, soybean meal organic matter digestibility was highest at 71% among all other tested products in this class.

Low-protein Plant

Among feedstuffs tested that are primarily included in diets for their carbohydrate fraction, energy digestibility was generally low. Rice bran had the highest energy digestibility at 60% and whole wheat had the lowest energy digestibility at 32%. Protein digestibility within this class of feedstuff ranged from 57% for Waxbar barley to 85% for whole wheat; moreover, barley protein digestibility was significantly lower than that of whole wheat or wheat flour. Organic matter digestibility was also low in these ingredients ranging from 36% for millrun wheat to 54% for both rice bran and wheat flour.

Discussion

Information on the apparent digestibility of protein and energy in extruded diets for rainbow trout is sparse. This lack of information often leads to extrapolation on the nutritive value of an ingredient based on the chemical composition and/or data on the digestibility of nutrients or energy in an ingredient when the diets were cold pelleted and did not undergo extrusion processing in a compound diet. Therefore, the current study was performed in extruded diets to mimic as closely as possible industry feed processing.

Examination of digestibility coefficients for protein and energy from feedstuffs used in extruded diets for trout appears to be warranted because of the fact that Cheng and Hardy (2003) noted reductions in protein digestibility in ingredients that were extruded prior to cold pelleting with fecal collection by settlement. These authors observed substantial effects of extrusion on protein digestibility in corn gluten meal and whole wheat that were reduced by 12 and 5.4 points, respectively, but only a 1-point reduction in barley protein digestibility and no effects on soy meal. Energy digestibilities also were significantly altered for corn gluten meal and whole wheat; extrusion increased the digestible energy coefficients by 8.9 and 23.1 points, respectively. Only limited further comparisons have been made regarding the effects of extrusion processing on nutrient digestibility of trout feeds. Stone et al. (2005) reported increased energy digestibility in extruded compound diets compared to cold-pelleted diets with no effects of diet processing on the apparent protein digestibility coefficients for the diets.

Other factors also may complicate protein digestibility comparisons across widely varying ingredients including nutrient inclusion level and fecal collection method. The basic assumption of the diet substitution method used for determining nutrient digestibility is that the reference diet nutrient digestibility does not change with the level of nutrient inclusion from the test ingredient. However, Aksnes et al. (1996) observed that while level of inclusion did not affect protein digestibility coefficients, increasing fishmeal levels did affect nitrogen free extract and this led to an overestimation of digestible energy. This observation would preclude the comparison of apparent digestible energy in ingredients having widely differing starch compositions. Therefore, we chose to statistically compare ingredients based on their similarities in proximate composition and source of origination. Another factor known to affect the comparability of digestibility data is the method of fecal collection. Austreng (1978) and Vandenberg and De la Noüe (2001) observed that the fecal stripping technique can underestimate protein and energy digestibility compared to the column collection technique outlined by Cho and Slinger (1979) or the automated feces collection devise used by Choubert et al. (1982). The hypotheses to explain underestimation by the fecal stripping technique include induction of defecation prior to complete digestion or contamination with other bodily fluids, while an overestimation may occur with the settlement techniques because of leaching of nutrients into the receiving water. The degree to which the stripping technique underestimates or, in contrast, the two fecal settlement techniques overestimate protein and energy digestibility is still debated. We chose to use the fecal stripping technique as a conservative estimate of digestibility of protein and energy.

Notwithstanding the previously described sources of variation in protein and energy digestibility, the values obtained in the current trial are comparable to protein and energy digestibility values previously reported for rainbow trout for ingredients high in protein (fish meals, animal by-products, and plant protein concentrates). Aksnes and Opstvedt (1998), Sugiura et al. (1998), Cheng and Hardy (2002), and Gomes et al. (1995) found that protein ADC ranges similar to the current trial in a variety of fish meals using cold-pelleted diets and all but Aksnes and Opstvedt (1998) used a fecal settlement technique.

Results observed for animal by-products protein and energy digestibility coefficients in the current trial also are comparable to literature values, and it appears that ingredient source may explain more of the variation in observed values for these ingredients than the technique used for determining the digestibility coefficient. Protein digestibility of blood meal (91%) was lower than the 97% observed for spray-dried blood products and was higher than that observed for blood products produced by other drying techniques by Bureau et al. (1999) who used fecal settlement and steam-pelleted diets. A comparison of feather meals also highlights potential variation in protein digestibility among ingredients. Bureau et al. (1999) tested four feather meals from different sources and found that hydrolyzed feather meal that had been steam tube dried had a higher protein digestibility (87%) than hydrolyzed feather meal dried by other methods. In the current trial, the protein digestibility of feather meal (87%) was comparable to values obtained by Bureau et al. (1999) and Sugiura et al. (1998), which used fecal settlement and cold-pelleted diet. Like feather meal, the protein digestibility of poultry by-product meal (PBM) often varies among sources. Digestibility of protein from pet food grade PBM in the current trial was 87% and was comparable to those values obtained for two poultry products tested by Bureau et al. (1999). Cheng and Hardy (2002), using fecal settlement and cold-pelleted diets, also noted variable protein digestibility of PBM meals in rainbow trout ranging from 83 to 87%, depending on grade. Dong et al. (1993), using the fecal stripping technique and cold-pelleted diets, reported lower values (64–78%) than that of the current trial, depending on PBM source; whereas Sugiura et al. (1998) observed a higher protein digestibility of 96% from PBM. Comparable trends in digestible energy values in the above studies also tend to follow the protein digestibility of the ingredients. Thus, discrepancies among protein digestibilities observed for any particular ingredient are ubiquitous in the literature, especially for products that are often variable in initial quality and composition. As previously noted, the quality of product going into the rendering process and the processing conditions can dramatically influence the final product's protein digestibility (Parsons et al. 1997; Wang and Parsons 1998).

Although bean varieties and methods used for concentrating soy proteins may vary, published ADCs are generally similar and high for the concentrates. Glencross et al. (2004, 2005) reported protein digestibility values of 98–107% for soy protein concentrate (SPC) and 98% for soy protein isolate in two trials with rainbow trout. Kaushik et al. (1995) reported 96% protein digestibility in trout fed SPC.

Corn gluten meal is another high-quality product based on protein digestibility. Yamamoto et al. (1998) observed an apparent protein digestibility coefficient of 95% for corn gluten meal, while Sugiura et al. (1998) observed a coefficient of 92% for the same product. On the other hand, Cheng and Hardy (2003) reported the digestibility of protein in corn gluten meal was only 75% when the ingredient was extruded prior to cold pelleting with the reference diet compared to 87% in cold-pelleted diets. Wheat gluten meal also exhibited high-protein digestibility in the current study, corroborating reports in the literature. For example, Glencross and Hawkins (2004) and Sugiura et al. (1998) also reported the digestibility of protein in wheat gluten was as high as 100%. RPC and BPC are relatively new feedstuffs with potential for use in fish feeds. Indeed, no comparative data are available for BPC and only one report exists for RPC in fish. Protein digestibility coefficients for both RPC and BPC were relatively high, at 89 and 92%, respectively, in the current study. Palmegiano et al. (2006) reported decreasing protein digestibility in diets as RPC concentrations increased relative to herring fish meal.

The fish meals, animal by-products, and plant concentrates data demonstrate that when high-protein ingredients are fed to rainbow trout, protein, and energy digestibility coefficients often parallel each other. Lipid content of the ingredient also influences the DE value even though most ingredients used in animal feed either have low lipid content (plant meals and concentrates) or have had the oils and fats partially extracted (fish meals and animal meals). This relationship was not observed when rainbow trout were fed the plant meals or low-protein plant ingredients from cereal grains. In the current trial, divergent protein and energy ADC values were obtained most notably in ingredients known to be high in fiber or have very high starch content. Thus, the comparability of ingredients/diets processed by cold pelleting or extrusion may be questionable at this juncture. It has been well documented that extrusion processing increases the gelatinization of the starch in an ingredient and thereby increased it digestibility to trout (Stone 2003). Difficulties also arise when using high starch ingredients as it has been noted that starch digestibility will vary with inclusion level in the diet (Krogdahl et al. 2005). It also appears that when plants are processed into concentrates or meals, an improvement in protein digestibility occurs. For example, soybean meal protein digestibility was 89% compared to 99% in soy protein concentrate. Even higher increases were seen in protein digestibility coefficients for the low-protein wheat products versus wheat gluten meal and corn versus corn gluten meal. Although unaddressed in the current trial, the reduced protein digestibility observed for the plant products in the current study may be because of increased rate of passage or interferences with the proteolytic enzymes in the gut lumen by the fiber or other carbohydrate fractions.

Conclusions

In the current trial, the fish meals and animal by-products exhibited high apparent protein and energy digestibility coefficients for rainbow trout in extruded feeds. The plant protein concentrates also exhibited high apparent protein and energy digestibility coefficients, particularly when compared to the plant meals of both high- and low-protein series. As plant-based diets are developed for rainbow trout and other fish species, it is apparent that further processing of the raw plant ingredients into concentrates or extrusion pelleting increases both the protein and the energy digestibility. These value-added products will have higher nutritional value for trout, and the data presented will assist in formulating trout diets on an equivalent digestible protein and energy basis.

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