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# *In Vitro* and *In Vivo* Feedstuff Digestibility for Snook, *Centropomus undecimalis*, Juveniles

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## Abstract

**The degree of hydrolysis (DH) of the ingredients was evaluated for *Centropomus undecimalis* juveniles. The *in vitro* experiment included wheat gluten (WG), corn gluten (CG), soybean meal (SBM) and pretreated phytase (SBM + phytase), soy protein concentrate (SPC), canola meal (CAN) and pretreated phytase (CAN + phytase), poultry byproduct meal (PBPM), chicken meal (CHKM), dried whey (DW), Pota meal (PM: mix of giant squid, fish soluble protein concentrate, crustacean meal, and fish oil), and Protiblend (PTB: aquatic and render mix). The highest acidic DH occurred with PTB (0.38 ± 0.06), CHKM (0.33 ± 0.3), and PBPM (0.25 ± 0.03). In the alkaline condition, PTB (1.6 ± 0.17 and 0.98 ± 0.05 for pyloric caeca and intestine, respectively) and CG (1.04 ± 0.4 and 0.75 ± 0.2 for caeca and intestine, respectively) provided the highest DH values. *In vitro* digestibility demonstrated that PTB was the highest (85.3%) followed by PBPM (51.4%), CAN + phytase (47.6%), CG (45.1%), and CHKM (46.5%). The *in vivo* experiment concerned the WG, CHKM, PBPM, PM, and PTB diets plus a reference diet with fishmeal (Ref diet). The total %DH was different ($P < 0.05$) with the lowest values for the WG diet (0.34 ± 0.09%) and Ref diet (0.34 ± 0.15). Free amino acid released during digestion was displayed for these diets and a bifactorial analysis produced no difference ($P > 0.05$). The apparent digestive coefficients ranged from 89.8 to 92.9% for protein and from 68 to 71.4% for energy.**

KEYWORDS

**amino acids, *in vitro* and *in vivo* digestibility, pH-stat, protein, snook**

The highest cost in finfish aquaculture is the feed, and the protein content represents the greatest proportion. Therefore, looking for alternative protein sources to replace traditional ones, such as marine sources (Córdova-Murueta and García-Carreño 2002), is a prerequisite for sustainable production. Among these parameters, the digestibility of the ingredients provides a primary idea of the body's ability to use them (Moyano and Savoie 2001; Alarcon et al. 2002; Barroso et al. 2002; Garcia-Lopez et al. 2003; Soria-Cuenca et al. 2013).

*In vitro* and *in vivo* methods for measuring protein utilization by fish were found to be

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complementary (Dimes et al. 1994a, 1994b). *In vitro* digestibility studies led to a determination of ingredient quality to evaluate possible savings and created a shortlist of potential sources (Ezquerra et al. 1997; Tibbetts et al. 2011). pH-Stat was previously used in research on human and fish screening among protein ingredients, and Pedersen and Eggum (1983) found reasonable accuracy in predicting digestibility. Based on this method, Dimes and Haard (1994) and Dimes et al. (1994a, 1994b) assessed salmonid feedstuffs. Later, Alarcon et al. (2002) and Silva et al. (2014) found a significant correlation between *in vitro* and *in vivo* digestibility for fish (Gomes Da Silva and Oliva-Teles 1998) to optimize protein fractions in a microencapsulated feed for marine fish larvae.

Meanwhile, several authors (Lee and Lawrence 1986; Smith and Tabrett 2008; Silva et al. 2014) worked on *in vivo* digestibility for marine fish to screen various ingredients and to formulate a grower feed using direct (gravimetric) or indirect (inert marker) methods (Cuzon et al. 1998).

*Centropomus parallelus* gut histological sections previously showed that proteins and lipids were absorbed mostly by the caeca, whereas only protein was absorbed in the rectum (Shimada et al. 2010).

The objective of the present study was to identify the most digestible ingredients among a series of feedstuffs to formulate a practical diet for the common snook, *Centropomus undecimalis*, and to be produced in controlled conditions.

## Materials and Methods

### *In Vitro* Digestibility

Fifteen snook were obtained from one spawning in the laboratory Unidad Multidisciplinaria de Docencia e Investigación de Sisal, UNAM, Mexico, and the larvae were reared according to Ibarra-Castro et al. (2011) to obtain juveniles fed on a commercial feed for their growout.

After the fish fasted for 48 h, they were transferred to the laboratory for analysis and kept in a 10-L container with seawater added with 0.1 mL clove oil/L as an anesthetic. Biometric measurements (total and standard length, max and min height, and weight) were recorded, and later, the fish were sacrificed to remove the stomach, caeca, and intestine. Each organ was deposited on ice in a beaker, placed in containers, and rinsed with distilled water. The organs were individually weighed and stored in bags at −80 C until ready for analysis.

Proximate analyses of the ingredients followed AOAC (1980) methods: this method consisted of nitrogen by elemental analysis based on direct and spontaneous sample combustion in an atmosphere of pure $O_2$ at 950–1400 C. CHN and S are transformed to $CO_2$, $N_2$, and $SO_2$ and carried by He to a single infrared cell with $CO_2$ and $SO_2$ are removed. Then, $N_2$ was measured by differential thermal conductivity; lipids were extracted by the Goldfish method and ash by incineration at 500 C in a muffle oven. The energy content of the ingredients was measured in an adiabatic calorimeter bomb (PARR, Moline, IL, USA), which was previously calibrated with benzoic acid.

To reduce the phytic acid content from soybean meal (SBM) and canola (CAN) meal, both ingredients were pretreated with β-propeller phytase (FTEII) designed to have high thermostability and activity over a broad range of pH (Viader-Salvadó et al. 2010) according to the method of Saunders et al. (1972) and modified by Dimes and Haard (1994).

Five enzymatic extracts from the digestive tract of three fish were individually prepared according to Silva et al. (2014). The stomach, pyloric caeca, and intestine were macerated in an Ultra Turrax® tissue homogenized with distilled water at 15:1 (15 mL/g tissue) and the mash was placed in 2-mL Eppendorf tubes and centrifuged at 16,170 *g* for 15 min at 4 C. Then, the middle layer was transferred to another Eppendorf tube and stored at −20 C.

Acidic and alkaline protease activities (Kunitz 1947, modified by Walter 1984) were obtained from enzyme extracts of the stomach, pyloric caeca, and intestine. For the acidic protease analysis, a 2-mL Eppendorf tube received hemoglobin (Hb) (1%) in glycine buffer and 0.1 M HCl to pH 2 and 5 µL of enzyme extract

was added and incubated for 5 min at 37 C. The reaction was stopped with 0.5 mL trichloroacetic acid (TCA) (20%) for 15 min at 4 C to precipitate the protein, the mixture was centrifuged at 13,370 *g* for 5 min at 4 C, and the supernatant absorbance was read at 280 nm.

For the alkaline protease analysis, a 2-mL Eppendorf tube received 0.5 mL casein (1%) at pH 9 and 0.5 mL of buffer Tris–HCl 100 mM + $CaCl_2$ 10 mM and 10 µL of enzyme extract and was incubated for 40 min at 37 C. The reaction was stopped with 0.5 mL TCA (20%) for 15 min at 4 C to precipitate the protein, the mixture was centrifuged for 5 min at 13,370 g, and the absorbance was measured at 280 nm; the enzymatic unit per milliliter was calculated as follows:

$$\frac{\text{U/mL} = \Delta\ \text{abs}_{280\text{nm}} \times \text{final volume of reaction (mL)}}{\text{CEM}_{\text{Tyrosine}} \times \text{time} \times \text{extracted volume (mL)}}$$

where CEM is the molar extinction coefficient, incubation time in minutes, and the amount of extract per milliliter.

Eleven ingredients were tested on a pH-Stat (Metrohm 842 Titrando) for DH determination on stomach (acidic), caeca, and intestine (alkaline) with a final volume of 5 mL distilled water and an equivalence of 8 mg protein/mL. The hydrolysis was conducted at 37 C for 15 min (stomach) and 45 min (caeca and intestine). The HCl 0.1 N maintained the pH 3.5 for acidic *in vitro* digestibility and NaOH 0.1 N was used for alkaline digestibility. Hb was used as a reference ingredient in the acidic digestibility and Hammerstein casein was used for alkaline digestibility. The unit number (tissue U/mL) for *in vitro* digestibility was displayed as follows: stomach (pool 1–3) 4611–5161, caeca (pool 1–3) 151–330, and intestine (pool 1–3) 26–188. The final volume of both HCl and NaOH served to determine that the $DH = h/h_{tot} \times 100$, where $h$ is the number of hydrolyzed peptide links and $h_{tot}$ is the total number of peptide links of the protein substrate. The number of hydrolyzed peptide links was calculated as $h = V_b \times N_b \times 1/\alpha \times 1/MP$, where $V_b$ is the volume of the base consumed (mL), $N_b$ is the normality of the base, α is the constant of dissociation for the α-$NH_2$ groups, and $M_P$ is the mass of the protein in the mix for the reaction.

To determine the DH of the different diets, 200 µL were sampled for acidic digestion at 0, 3, and 15 min and for alkaline digestion at 0, 30, and 45 min. These samples were mixed with an equal 12% TCA volume and then stored at −80 C for subsequent tests such as an analysis of total amino acids by electrophoresis (Izquierdo et al. 2001; Silva et al. 2014).

The free amino acid (FAA) analysis is based on the reaction with the α-amino o-phthalaldehyde (OPA) and β-mercaptoethanol OPA solution, which was mixed with 25 mL of sodium tetraborate (100 mM), 2.5 mL of 20% sodium dodecyl sulphate, 40 mg of OPA dissolved in 1 mL methanol, 100 µL of β-mercaptoethanol, and 50 mL pyrogen-free water prepared daily. The 25-µL samples plus 1 mL OPA solution were briefly mixed and incubated for 2 min at room temperature, and the absorbance was read at 340 nm. The total amino acid analysis (TAAA) (mg/mL) of the reaction referred to a standard curve with L-leucine (0.5 mg/mL) for the 200-µL sample of acidic digestion at 0.7 (15 min) and in alkaline digestion at 0.3 (45 min). Blind samples for intestine occurred at three time points (0, 30, and 45 min) and were mixed with an equal volume of 12% TCA and stored at −80 C. This procedure was repeated in triplicate for each diet (Church et al. 1983).

### *In Vivo* Digestibility

A total of 180 juveniles with a 45-g mean wet weight were placed in eighteen 100-L tanks (10 fish/tank) in a closed recirculation system with controlled parameters. A randomized experimental design with six treatments and three replicates per treatment was used. The fish were fed 3% of their biomass daily at 0900, 1300, and 1800 h (Garcia-Galano et al. 2003).

Six diets were prepared according to Silva et al. (2014); (Table 1). Raw materials were screened at 250 µm and mixed for 10–15 min. The oil was mixed for 10–15 min, and the binder was gelatinized by adding boiling water and stirring until the dough was extruded through a meatmincer to form pellets, which were stored

TABLE 1. *Diet composition for the* in vivo *digestibility experiment of juvenile* Centropomus undecimalis.[1]

| Ingredients | Reference diet | Wheat gluten diet | Pota meal diet | Chicken meal diet | Soybean meal diet | Protiblend diet |
|---|---|---|---|---|---|---|
| Wheat gluten Viten[2] | | 30 | | | | |
| Poultry byproduct meal[3] | | | 30 | | | |
| Chicken meal[3] | | | | 30 | | |
| Giant squid meal[4] | | | | | 30 | |
| Protiblend[5] | | | | | | 30 |
| Anchovy meal[6] | 70 | 49 | 49 | 49 | 49 | 49 |
| Wheat flour[6] | 21 | 15 | 15 | 15 | 15 | 15 |
| Cod liver oil[7] | 3 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 |
| Soy lecitin[7] | 1 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| Min + vit premix[8] | 2 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 |
| Carboxymethyl-cellulose[10] | 1 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| Zeolite[9] | 1.5 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
| Crude protein | 63.75 | 61.74 | 55.3 | 59.62 | 60.36 | 60.23 |
| Fat | 9.47 | 13.87 | 13.33 | 13.6 | 11.06 | 12.71 |
| Carbohydrate | 13 | 9 | 9 | 9 | 9 | 9 |
| Ash | 9.13 | 10.13 | 12.96 | 12.29 | 12.78 | 14.67 |
| Gross energy (kJ/g) | 9.7 | 12.3 | 11.1 | 11.0 | 10.1 | 11.4 |

[1]The results are expressed in % wet weight using the AOAC (1980) method for proximate analysis.
[2]Roquette Frères S.A., Francia.
[3]MaltaCleyton S.A. de C.V., Mérida, Yucatán, Mexico.
[4]IMPEXCO, Peru.
[5]Soluciones Integrales de Nutrición SA de CV, Mexico.
[6]Productos Químicos de Yucatán S.A. de C.V., Mérida, Yucatán, Mexico.
[7]Proteínas y Oleicos S.A. de C.V., Mérida, Yucatán, Mexico.
[8]DMS Nutritional Products Mexico S.A. de C. V., Mexico.
[9]Wachsen Industry Co., Ltd., Quingdao, China.
[10]Comercializadora Mayorista del Golfo S.A. de C.V., Mérida, Yucatán, Mexico.

TABLE 2. *Free amino acid concentrations (*μ*g/mL) released from acidic and alkaline hydrolysis using 268 multi-enzymatic extracts of the juvenile* Centropomus undecimalis *on protein ingredients (mean ± ES,* n = *3): reference diet (Refd), wheat gluten diet (WGd), chicken meal diet (CHKMd), poultry byproduct diet (PBPd), Pota meal diet (PMd), and Protiblend diet (PTBd).*[1]

| | Stomach | | | | Caeca | | | Intestine | |
|---|---|---|---|---|---|---|---|---|---|
| Diet | $t_0$ | 6 min | 15 min | $t_0$ | 30 min | 45 min | $t_0$ | 30 min | 45 min |
| Refd | $1.4 \pm 0.2^{a}$ | $1.8 \pm 0.04^{a}$ | $1.7 \pm 0.1^{a}$ | $0.23 \pm 0.1^{ab}$ | $2.3 \pm 0.5^{a}$ | $2.3 \pm 0.3^{a}$ | $2.2 \pm 0.2^{a}$ | $3.7 \pm 0.6^{a}$ | $3.3 \pm 0.3^{a}$ |
| WGd | $0.2 \pm 0.04^{c}$ | $0.05 \pm 0.1^{c}$ | $0.4 \pm 0.07^{c}$ | $0.26 \pm 0.3^{b}$ | $1.2 \pm 0.3^{b}$ | $1.2 \pm 0.2^{a}$ | $0.7 \pm 0.3^{b}$ | $2.4 \pm 0.5^{a}$ | $2.4 \pm 0.4^{a}$ |
| CHKMd | $0.8 \pm 0.06^{b}$ | $1.05 \pm 0.08^{ab}$ | $1.2 \pm 0.08^{ab}$ | $0.9 \pm 0.2^{ab}$ | $1.9 \pm 0.00^{a}$ | $2.2 \pm 0.3^{a}$ | $1.44 \pm 0.5^{ab}$ | $3.3 \pm 0.6^{a}$ | $3.0 \pm 0.5^{a}$ |
| PBPd | $0.9 \pm 0.1^{b}$ | $1.1 \pm 0.2^{ab}$ | $1.2 \pm 0.2^{ab}$ | $1.03 \pm 0.6^{ab}$ | $2.6 \pm 0.7^{ab}$ | $2.8 \pm 0.9^{ab}$ | $1.4 \pm 0.6^{ab}$ | $3.2 \pm 0.4^{a}$ | $3.3 \pm 0.4^{a}$ |
| PMd | $0.9 \pm 0.2^{b}$ | $1.05 \pm 0.03^{bc}$ | $1.1 \pm 0.1^{bc}$ | $1.45 \pm 0.3^{a}$ | $2.5 \pm 0.3^{a}$ | $2.5 \pm 0.4^{ab}$ | $1.2 \pm 0.4^{ab}$ | $3.3 \pm 0.7^{a}$ | $3.6 \pm 1.3^{a}$ |
| PTBd | $0.8 \pm 0.03^{b}$ | $0.96 \pm 0.0bc^{a}$ | $1.03 \pm 0.09^{bc}$ | $1.11 \pm 0.1^{a}$ | $2 \pm 0.1^{ab}$ | $2.0 \pm 0.3b^{a}$ | $1.44 \pm 0.4^{ab}$ | $3.2 \pm 0.4^{a}$ | $3.3 \pm 0.6^{a}$ |

[1]Different letters in superscript indicate significant differences ($P < 0.05$).

at 40 C (Table 2). To quantify apparent digestive coefficient of ingredient ($ADC_{ingredient}$) and ADC, the fish fasted for 48 h and were then fed. The collection of feces started 2 d after the feeding started (Borquez and Cerqueira 1998; Wainwright et al. 2006). Daily and before each feeding, the waste was removed from the tanks by siphoning using a 3-mm-diameter plastic tube. After feeding (30 min later), unconsumed feed was also removed from the tanks. The feces were removed each hour between each feeding by siphoning using a plastic tube and cellulose paper. After daily collection, the feces were washed with distilled water and dried at 60 C.

A randomized design included six treatments with three replicates. $ADC_{DM}$ and

TABLE 3. *Proximal analysis of the ingredients used in this study.*

| Ingredient | Moisture | Ash | EE | CP | NFE | Cellulose | GE kJ/g |
|---|---|---|---|---|---|---|---|
| Chicken meal | 3.93 | 12.46 | 12.64 | 66.85 | | – | 16 |
| Poultry byproduct | 3.81 | 16.73 | 14.73 | 62.47 | 1 | – | 20.3 |
| Protiblend | 4.90 | 14.54 | 8.45 | 68.77 | | – | 15 |
| Pota meal | 7.84 | 6.62 | 14.01 | 55.26 | 16 | – | 15 |
| Dried whey | 2.47 | 7.85 | 0.22 | 12.84 | 48 | – | 16 |
| Corn gluten | 5.04 | 1.56 | 2.35 | 69.19 | 22 | 1.5 | 19.8 |
| Wheat gluten | 6.78 | 0.90 | 1.76 | 80.82 | | – | 22 |
| Canola meal | 4.86 | 7.10 | 1.72 | 44.77 | 41 | 13 | 17 |
| SBM | 7.01 | 6.95 | 1.77 | 51.21 | 33 | 3.5 | 18.9 |
| Soy protein concentrate | 4.80 | 6.79 | 0.23 | 66.66 | 22 | 2 | 19 |
| SBM + phytase | 7.01 | 6.95 | 1.77 | 51.21 | 33 | 3.5 | 18.9 |
| Canola meal + phytase | 4.86 | 7.10 | 1.72 | 44.77 | 41 | 13 | 17 |

CP = crude protein; EE = ether extract; GE = gross energy; NFE = nitrogen free extract; SBM = soy bean meal.

$ADC_{proteín}$ (DM = dry matter) were measured using zeolite as a marker (silica as a frustule component of diatomaceous earth), and samples were incinerated in a muffle furnace with crucibles to reach 500 C. The six diets included a known amount, and the feces were collected to calculate the following formula: $ADC_{DM} = 100 \times (1 - (\%$ zeolite diet/% zeolite in feces); $ADC_{proteín} = 100 \times (1 - (\%$ zeolite diet/% zeolite feces) (% crude protein feces/% crude protein diet); and $ADC_{energy} = 100 \times (1 - (\%$ zeolite diet/% zeolite feces) (energy feces/energy diet). $ADC_{ingredient} = ADC_{test\ diet} + ([ADC_{test\ diet} - 0.7 \times ADC_{ref\ \ diet}] \times [0.7\ \ D_{ref}\ /0.3\ \ D_{ing}])$, where $D = \%$ CP (Bureau and Hua 2006).

### *Statistical Analysis*

For the differences between the DH values of the ingredients, the DH of the diets, and the ADC coefficients of the diets and ingredients, the one-way ANOVA was used. The FAA values were analyzed using a bifactorial ANOVA to consider the time of sampling and treatments as factors. When significant differences were observed, the Tukey multiple range test was used to detect a difference at the 5% confidence level. If homogeneity of the variance was not met, a Kruskal–Wallis test was used and the results reported as median ± quartile range. Lineal regression was calculated to correlate the DH of the ingredient and the apparent digestibility coefficients.

## Results

The proximate composition ranges of the ingredients were as follows: moisture (2–8%), crude protein (13–69%), ether extract (0.2–15%), and ash (1–17%) (Table 3).

The *in vitro* acidic digestibility of the plant protein ingredients was lower than those from an animal origin (from $0.05 \pm 0.01$ for wheat gluten [WG] to $1.04 \pm 0.4$ for corn gluten [CG] compared to $0.6 \pm 1.5$ for Pota meal [PM] and $1.6 \pm 0.17$ for Protiblend [PTB]; Table 4).

The *in vitro* alkaline digestibility results from the pyloric caeca showed that PTB meal (PTBM) ($1.6 \pm 0.1$) achieved the highest value ($P < 0.05$). For the intestine, the highest DH value was also obtained for PTBM ($1 \pm 0.04$). The lowest significant values in both the pyloric caeca and the intestine were obtained with WG meal (Table 4).

The total DH values of each feedstuff indicated that PTBM had the highest value ($2.99 \pm 0.29\%$), which represented $85.3 \pm 4.5\%$ compared to the reference Hb/casein. The best plant protein ingredients were CAN + phytase ($1.76 \pm 0.3$ and $46.5 \pm 9.2\%$) and CG meal ($1.6 \pm 0.2$ and $45.1 \pm 6\%$). Similarly, total DH was obtained in poultry byproduct (PBP) meal ($1.9 \pm 0.5$ and $51.4 \pm 1.5\%$) and chicken meal (CHKM) ($1.72 \pm 0.3$ and $46.5 \pm 9.2\%$).

The DH of the diets formulated for the *in vitro* digestibility experiment showed significant differences in the stomach and pyloric caeca, where the lowest significant value was obtained in the WG diet and the highest value in the PTB diet (Table 5). For the intestine, the values

TABLE 4. *Degree of hydrolysis (DH) for various sources of protein using enzymatic extracts from the stomach (according to a one-way ANOVA, mean ± SE); the pyloric caeca and intestine of juveniles of* Centropomus undecimalis *(according to Kruskal–Wallis test; median ± quetile range).*[1]

| | Stomach | Caeca | Intestine | Hemoglobin/casein | |
|---|---|---|---|---|---|
| | DH | | | DH total | %[2] |
| Hemoglobin/casein | $0.04 \pm 0.006^{b}$ | $0.64 \pm 0.08^{a}$ | $0.37 \pm 0.2^{a}$ | $3.69 \pm 0.0^{a}$ | |
| Soybean meal (SBM) | $0.024 \pm 0.05^{b}$ | $0.05 \pm 0.02^{ef}$ | $0.11 \pm 0.07^{ef}$ | $0.97 \pm 0.2^{de}$ | $26.2 \pm 4.6^{c}$ |
| SBM + phytase[3] | $0.002 \pm 0.005^{b}$ | $0.64 \pm 0.1^{def}$ | $0.35 \pm 0.009^{def}$ | $1.05 \pm 0.04^{de}$ | $28.3 \pm 1^{c}$ |
| Soy protein concentrate[4] | $0.009 \pm 0.01^{b}$ | $0.8 \pm 0.08^{bc}$ | $0.34 \pm 0.1^{ef}$ | $1.3 \pm 0.4^{cde}$ | $35.7 \pm 11^{c}$ |
| Canola meal | $0.027 \pm 0.02^{d}$ | $0.8 \pm 0.2^{cde}$ | $0.18 \pm 0.18^{f}$ | $1.16 \pm 0.07^{de}$ | $31.5 \pm 1.9^{c}$ |
| Canola + phytase | $0.0097 \pm 0.01^{d}$ | $0.9 \pm 0.6^{bc}$ | $0.7 \pm 0.7^{c}$ | $1.76 \pm 0.5^{cd}$ | $47.6 \pm 14^{b}$ |
| Corn gluten | $0.005 \pm 0.01^{bc,d}$ | $1.05 \pm 0.15^{b}$ | $0.75 \pm 0.3^{c}$ | $1.6 \pm 0.2^{cd}$ | $45.1 \pm 6^{b}$ |
| Wheat gluten[5] | $0.057 \pm 0.008^{b}$ | $0.013 \pm 0.007^{f}$ | $0.02 \pm 0.01^{f}$ | $0.18 \pm 0.0^{f}$ | $4.87 \pm 0.01^{d}$ |
| Protiblend[6] | $0.38 \pm 0.06^{a}$ | $1.6 \pm 0.16^{a}$ | $1 \pm 0.04^{b}$ | $2.99 \pm 0.2^{b}$ | $85.3 \pm 4.5^{a}$ |
| Chicken meal[7] | $0.08 \pm 0.02^{b}$ | $0.45 \pm 0.14^{bc}$ | $0.45 \pm 0.15^{d}$ | $1.72 \pm 0.3^{cd}$ | $46.5 \pm 9.2^{b}$ |
| Dried whey | $0.33 \pm 0.3^{ab}$ | $1.02 \pm 0.3^{bcd}$ | $0.38 \pm 0.2^{ef}$ | $1.35 \pm 0.4^{cde}$ | $36.5 \pm 11^{c}$ |
| Poultry byproduct[7] | ND | $1.05 \pm 0.07^{b}$ | $0.6 \pm 0.2^{de}$ | $1.9 \pm 0.05^{cd}$ | $51.4 \pm 1.5^{b}$ |
| Pota meal[8] | $0.25 \pm 0.03^{a}$ | $0.6 \pm 0.16^{def}$ | $0.21 \pm 0.1^{f}$ | $00.84 \pm 0.2^{e}$ | $22.8 \pm 5.8^{c}$ |

ND = none detected.

[1]The *in vitro* digestibility (%) of the ingredients was examined in relation to a hemoglobin/casein standard (according to a one-way ANOVA, mean ± SE).

[2]% Digestibility relative to hemoglobin/casein total hydrolysis.

[3]In this work, the beta-propeller phytases (FTEII) were designed to have high thermostability and activity over a broad range of pH (Viader-Salvadó et al. 2010) and were used for pretreatment of both the soybean and canola meal.

[4]Profine, Chile.

[5]Roquette frères, France.

[6]Mix of aquatic and terrestrial animals produced by Soluciones Integrales de Nutrición, SA de CV, Mexico.

[7]Proteínas Marinas y Agropecuarias SA de CV, Mexico.

[8]A mix of giant squid meal, dehydrated fish soluble protein concentrate, crustacean meal, and fish oil and produced by Animal Feed SA de Cv, Mexico.

TABLE 5. *The degree of hydrolysis (DH) (acid, alkaline, and total DH%) and % digestibility of the diets using multi-enzymatic extracts of juveniles of* Centropomus undecimalis*: reference diet (Refd), wheat gluten diet (WGd), chicken meal diet (CHKMd), poultry byproduct diet (PBPd), Pota meal diet (PMd), and Protiblend diet (PBTd).*[1,2]

| | DH | | | | |
|---|---|---|---|---|---|
| Diet | Stomach | Pyloric caeca | Intestine | Total | Digestibility% |
| Hemoglobin/casein | $0.06 \pm 0.02^{b}$ | $0.20 \pm 0.06^{a}$ | $0.26 \pm 0.06^{a}$ | $0.57 \pm 0.10^{a}$ | |
| Refd | $0.08 \pm 0.06^{b}$ | $0.08 \pm 0.06^{ab}$ | $0.13 \pm 0.04^{a}$ | $0.34 \pm 0.10^{b}$ | $51 \pm 2.0^{a}$ |
| WGd | $0.17 \pm 0.06^{a}$ | $0.04 \pm 0.02^{b}$ | $0.17 \pm 0.08^{a}$ | $0.34 \pm 0.09^{b}$ | $51 \pm 1.1^{a}$ |
| Chkd | $0.2 \pm 0.19^{a}$ | $0.10 \pm 0.03^{a}$ | $0.17 \pm 0.08^{a}$ | $0.5 \pm 0.2^{ab}$ | $67 \pm 3.5^{a}$ |
| PBPd | $0.18 \pm 0.13^{a}$ | $0.08 \pm 0.06^{ab}$ | $0.17 \pm 0.06^{a}$ | $0.34 \pm 0.10^{ab}$ | $61 \pm 5^{a}$ |
| PMd | $0.23 \pm 0.16^{a}$ | $0.05 \pm 0.02^{b}$ | $0.14 \pm 0.07^{a}$ | $0.42 \pm 0.09^{ab}$ | $60 \pm 4.2^{a}$ |
| PBTd | $0.18 \pm 0.06^{a}$ | $0.10 \pm 0.04^{a}$ | $0.17 \pm 0.03^{a}$ | $0.45 \pm 0.07^{ab}$ | $64 \pm 3.5^{a}$ |

[1]The results are presented according to a one-way ANOVA (mean ± SE).

[2]Different letters in superscript indicate significant differences ($P < 0.05$).

were similar ($P > 0.05$). For the total DH value of the diets, the lowest values corresponded to the WG diet and reference diet (Ref diet). The digestibility percentages of the diets were not significantly different ($P > 0.05$; Table 5).

FAA released in the acidic condition differed in all three time points (0, 6, and 15 min). The Ref diet (fishmeal) had the highest values of 1.8 and 1.7 at 6 and 15 min, respectively, followed by PBP (1.1 and 1.2, respectively) and CHKM (1.1 and 1.2, respectively). The diet containing French WG Viten® had the lowest amount of amino acids released (0.05 and 0.4 at 6 and 15 min, respectively; Table 2). Under

TABLE 6. *Apparent digestive coefficient (ADC) % for dry matter (DM), protein, and energy of* Centropomus undecimalis *(mean ± SE).*[1]

| | $ADC_{DM}$ | $ADC_{protein}$ | $ADC_{energy}$ |
|---|---|---|---|
| Reference diet | $67.8 \pm 3.2^{ab}$ | $91.6 \pm 0.5^{a}$ | $85.5 \pm 1.8^{b}$ |
| Wheat gluten diet | $65.8 \pm 3.4^{ab}$ | $89.9 \pm 1.7^{ab}$ | $82.2 \pm 3^{b}$ |
| Chicken meal diet | $74.2 \pm 5.6^{a}$ | $90.9 \pm 2^{ab}$ | $90.5 \pm 0.8^{a}$ |
| Poultry byproduct diet | $70.9 \pm 6.1^{ab}$ | $92.9 \pm 1.5^{a}$ | $82.8 \pm 2^{b}$ |
| Pota meal diet | $59 \pm 4.1^{b}$ | $87.2 \pm 1.1^{b}$ | $77.3 \pm 2.2^{c}$ |
| Protiblend diet | $63.9 \pm 5.3^{ab}$ | $90.5 \pm 1.3^{ab}$ | $85 \pm 2.5^{b}$ |

[1]Different letters in superscript indicate significant differences ($P < 0.05$).

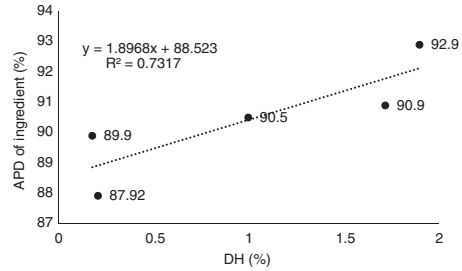


FIGURE 1. *The correlation between apparent protein digestibility (APD, %) and* in vitro *degree of protein hydrolysis (DH %) with enzymatic extracts of juveniles of* Centropomus undecimalis. *Regression not significant (*P = *0.06).*

the alkaline conditions, there were no differences at 45 min for the protein ingredients whether from the caeca (1.2–2.8) or from the intestine (2.4–3.6).

In relation to the *in vivo* digestibility, significant differences in $ADC_{DM}$ were found among the ingredients ($P < 0.05$). The lowest value was obtained in the PM diet (59%), which was similar to the Ref diet (Table 6). $ADC_{protein}$ discriminated between animal ingredients with the lowest value found in PM ($P < 0.05$; Table 6). PBP (93%) was more digestible than CHKM (91%). PBP meal and quality WG were similarly ranked (91 and 90%), which contrasted with the pH-Stat results (Table 6). PM was significantly less digestible than all of the other ingredients based on its ADC. However, no significant differences were observed in relation to the $ADC_{ingredients}$ ($P > 0.05$; Table 6).

The correlation between the apparent protein digestibility and the degree of hydrolysis of the five ingredients is shown in Figure 1; the regression was not significant ($P > 0.05$).

## Discussion

To the best of our knowledge, this study is the first description of the potential for digestion (*in vitro*) and absorption (*in vivo*) of plant and animal ingredients for juvenile *C. undecimalis*. Centropomidae is a family with several species that are good candidates for aquaculture such as snook (Tucker 1987; Zarza-Meza et al. 2006; Tsuzuki et al. 2007) and Asian sea bass (Katersky and Carter 2007). However, the level of knowledge varies from one species to another. *Lates calcarifer* is known for its capacity to digest SBM as a replacement for fishmeal (Boonyatpalin 1998) and for its digestible protein/digestible energy (DP/DE) requirement. In all three presentations, SBM gave the same DH but the concentrate (soy protein concentrate [SPC]) contained an antinutritional factor (Kunitz 1947; Garcia-Carreno et al. 1997), which could explain an absence of differences between the meal and the concentrate. *C undecimalis* juveniles were tested at the beginning of their growth potential when their ability to digest dry ingredients was low.

The results of acidic and alkaline protease activities showed high levels in the stomach (acidic) and intestine (alkaline), but the caeca activity remained low, which was similar to previous results reported in snook (Concha 2008). Snook is a colossal carnivorous fish and the main hydrolysis occurs in the stomach while the final hydrolysis is performed in the caeca and intestine, where freed nutrients are available for absorption (mostly in the intestine). The *in vitro* digestibility in the stomach had a higher DE for various alternative protein sources, such as marine and terrestrial animal flour (PTB), as well as land animal protein (chicken feed grade and chick meal); these results indicate that there are potential alternative protein sources to fishmeal or other marine sources to make feeds (Cho 1992), which meet the nutritional requirements of *C. undecimalis*. However, PM showed a lower value than expected (50%), which was probably due to processing, a finding that has been previously encountered for shrimp

(Ezquerra et al. 1997; Córdova-Murueta and García-Carreño 2002).

The plant ingredients produced lower DH values than the control (Hb), which is also indicative of the carnivorous lifestyle of this species. Alkaline digestibility was divided in two parts: the first focused on the pyloric caeca and the second on the intestine. This test showed slight differences when compared to acidic digestibility. Casein in both cases had the highest DH; the only source of marine origin (PTB) had a lower DH compared to plant ingredients (CAN, CAN + phytase, or CG). The DH from acid and alkali resulted in high % digestibility for PTB, PBP meal, and CHKM; in the case of animal protein, the digestibility is known to be closest to 100%, but plant-derived ingredients, such as CG and CAN + phytase, also produced values close to 100%. The DH discriminated between ingredients and for the same ingredients, regardless of whether phytase was added or not (Table 4).

Variations in the DH from different parts of the fish gut were clear when pretreated phytase + CAN and SBM were tested. In the stomach, the extract had no effect on both meals containing phytase. In the caeca and intestine, the phytase used on CAN produced higher DH values than with SBM (Table 4), which showed that an exogenous enzyme had varying impacts according to the nature of the ingredients fed to the snook.

A comparison of the ADC values (Gomes Da Silva and Oliva-Teles 1998; Lemos et al. 2009) within the Centropomidae family revealed 72% with Danish fishmeal in snook and 88 and 83% in Asian seabass for protein and energy, respectively (Williams et al. 2003); $ADC_{protein}$ and $ADC_{energy}$ for SBM was 86 and 69%, respectively. These data show an optimal DP/DE ratio that is fundamentally dependent on digestibility results; with marine fish, only data on Centropomidae were produced by means of the three experiments testing constant DP level, constant energy level, or the constant ratio in *L. calcarifer*. This information permitted creation of a commercial formulation in Singapore to farm seabass (Lee et al. 1995).

In relation to SPC, an antitrypsic factor could concentrate and then alter the protein digestibility results in trout (Escaffre et al. 2007; Sarker et al. 2011) and snook (Grabner and Hofer 1985), which could explain an absence of differences between the meal and the concentrate for soybean.

The ingredients were treated for *in vitro* or *in vivo* digestibility while considering primarily the protein content. From all of the potential ingredients, PTB had a high digestibility (DH) because it is a mix of shrimp meal and meat meal. Therefore, the chitin could have been partly digested (amino sugars β 1–4 bounds). What benefit could Centropomidae retrieve from plant sources? Native WG produced a high DH and $ADC_{protein}$. CAN and SBM (meal or concentrate) were equally digested. Therefore, despite a preference for carnivory, Centropomidae maintained a level of digestibility for plant sources in the following order: WG > SBM > CAN > SPC. However, the results did not correlate except for WG and CHKM. The question was also raised for squid (Córdova-Murueta and García-Carreño 2002) that can be ingested by *C. undecimalis* in its environment; its native protein had a high digestibility as well as the native gluten.

The predictive role of DH of the best ingredients using enzymatic extracts of *C. undecimalis* juveniles was verified through the high positive correlation between the apparent protein digestibility ($r^2 = 0.7$); however, the regression was not significant ($P > 0.05$) although all of the values were higher than 80% as was indicated by Lemos et al. (2009).

Hydrolysis-produced FAAs were released during the *in vitro* test and did not indicate differences among the parts of the digestive tract ($P > 0.05$). The stomach did not discriminate between WG and other sources after 15 min and this absence of a difference was maintained in the intestine; all of the other sources released equal amounts of amino acids after 45 min in the intestine. This indicated a potentially similar efficiency for tissue buildup regardless of the protein sources.

Therefore, the information on digestibility of this fish species can be summarized in a putative formula for a grower feed in the % fed: PTB, 15; chick meal, 15; chicken feed grade, 10; squid, 5; WG, 5; SBM, 10; SPC, 10; CAN,

10; and premix, 10 to provide a feed with 42% crude protein (CP) (34% DP). From an economics viewpoint, another Centropomidae, *L. calcarifer*, was studied with two main protein sources (fishmeal and regular SBM) to reach a balanced DP/DE (33 mg protein/kJ), which met the requirement of juveniles with 1.5 g balanced digestible protein per 64 kJ for an specific growth rate at 2.4% (Lee et al. 1995). This preliminary study opens the way for further research with native species to adapt the formulation of feed to their digestive potential and then examine the final cost of feed to produce common snook in floating cages or earthen ponds (Zarza-Meza et al. 2006) along the coast of the Yucatan.

## Conclusions

Generally, juvenile *C. undecimalis* digested and absorbed animal protein sources (marine and/or terrestrial) more effectively. PTB is highlighted, and, aside from PBP and CHKM, only CG was effective among the plant sources. The selection of available plant-based ingredients should be considered not only on the basis of proximate composition but also for soluble fiber and cellulose content. This content is often pooled in NFE to discriminate among CAN, soya, soluble distiller's grain, and millrun and should be considered to formulate on a low-cost basis as well as on the digestive physiology of *C. undecimalis.*

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