Technical Note

Evaluation of Industrial Sub-Product-Based Diet Alternatives to *P. vannamei* Culture Using pH-Stat

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INTRODUCTION

The culture of white shrimp *Penaeus vannamei* constitutes the world's largest aquaculture activity, with 5% annual growth. (FAO, 2020). One of the main limiting factors to the culture of this species is the high cost of balanced feeds, accounting for 50-70% of the production costs. (Hamidoqhli *et al*., 2020).

The high prices of feds in the international market have stimulated an alternative production of feeds that meet the conventional feedstuff requirements. In Cuba, the availability of several industrial sub-products with nutritional value may be used to design symbiotic diets (Martín, 2009).

Protein digestibility through pH-stat, using enzymes from shrimp hepatopancreas, was described and linked to the seeming digestibility in *Penaeus vannamei* juveniles, by Ezquerra et al. (1997). A high correlation of *in vitro* digestibility has been observed in pond-growing shrimps (Lemos and Nunes, 2008). This technique was used to evaluate the *in vitro* digestibility of some industrial sub-products, such as beer barley husks, wheat husks, and shrimp heads. Then it was compared with the digestibility offered by conventional feedstuffs to evaluate diet proposals based on industrial sub-products for *P. vannamei* culture through the pH-stat method.

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DEVELOPMENT

The *in vitro* digestibility of beer barley husks, wheat husks, Exia feedstuff (30%) samples (Biomar®, Ecuador: crude protein, 30%, ethereal extract: 5%, crude fiber: 8%, digestible energy: 2 985 Kcal/kg), and feedstuff for the parents (40%) (Biomar®, Ecuador: crude protein, 40%, ethereal extract: 5%, crude fiber: 16%, digestible energy: 2985 Kcal/kg). The results were used to compare the conventional feedstuffs with a new feed alternative based on industrial sub-products.

The diet, consisting of beer barley husks and shrimp heads, was ground using a hammerhead mill on a 500-micron sieve. Each sample was weighed to reach a 640 mg protein concentration in 80 mL of distilled water, then they were homogenized for 45 minutes, pH 7.9.

The hepatopancreas from 50 fattening shrimps were removed and homogenized under low temperatures, then they were centrifuged at 3000 x g and the supernatant was collected. The enzymatic activity curve was determined by azocasein and protease reaction (Lemos and Nunes 2008) from the hepatopancreas, considering that,

$$AE = \left(\frac{\Delta DO}{\Delta t}\right) * \left(\frac{1}{k}\right) * \left(\frac{Vens}{Venz}\right)$$

AE: Enzymatic activity.

Vens: assay volume

Venz: enzyme volume

ΔDO: absorbance variation.

Δt: time variation.

K.azo= 9656 mol/L-1

The *in vitro* ingestion reaction was made using 4U/ml hepatopancreas protease consisting of 125 ml of the hepatopancreas extract compared to the commercial feedstuff. The pH was adjusted to 8.0, with $NaOH^-$ 0.1 M.

The feed sample analysis was analyzed using the pH-stat method (Lemos and Nunes, 2008).

$$DH\% = B * Nb * \frac{1}{\alpha} * \frac{1}{Mp} * \frac{1}{Htot} * 100$$

Where:

DH%: *In vitro* digestibility

B: NaOH vol

Nb: NaOH normality

Mp: protein quantity

Htot: equivalent to peptide bonds.

1/α: pK, amino groups

The beer barley husks were found to have greater digestibility than the wheat husks (P<0.05) (Figure 1).

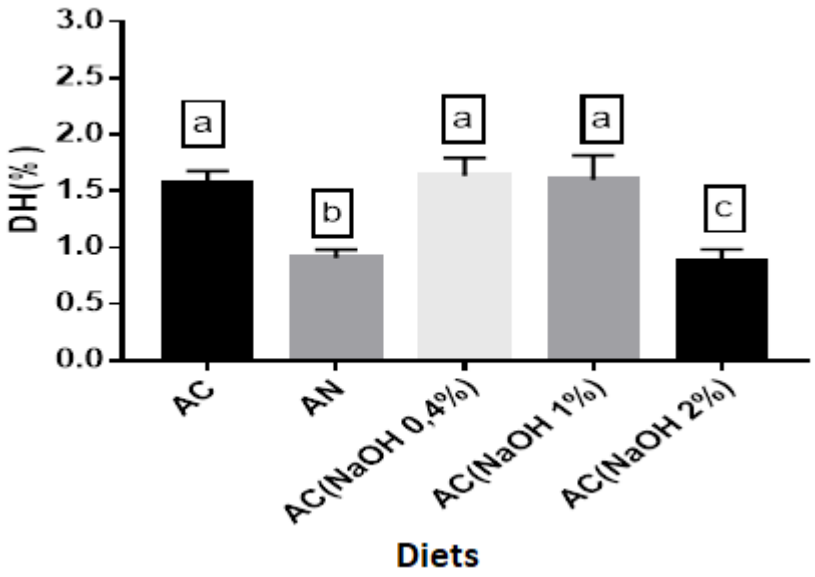

Figure 1 DH (%) In vitro digestibility of AC: beer barley husks, AN: wheat husks. AC NaOH (beer barley husks treated with 0.4, 1, and 2% NaOH to degrade fiber and improve digestibility).

Pre-treatment of beer barley husks with NaOH kept *in vitro* digestibility of the diets (P>0.05).

The combination of beer barley husks with yeast and shrimp heads (Diet 1) was observed to have similar digestibility to the commercial feed (P>0.05) (Figure 2).

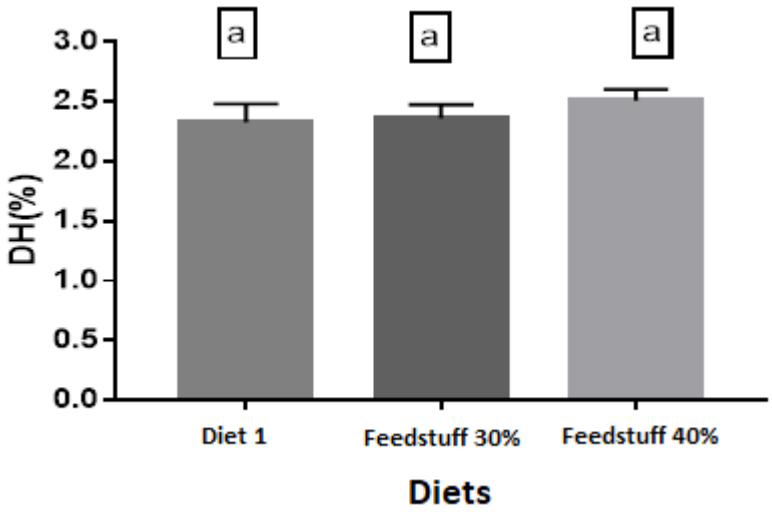

Figure 2. DH (%) *In vitro* digestibility of the symbiotic diet and commercial feedstuffs (Exia feedstuff (30 and 40%, Biomar®, Ecuador: crude protein, 30%, ethereal extract: 5%, crude fiber: 8%, digestible energy: 2 985 Kcal/kg and crude protein, 40%, ethereal extract: 5%, crude fiber: 16%, digestible energy: 2985 Kcal/kg respectively). Diet 1: Beer barley husks, shrimp heads, and yeast.

The digestibility of symbiotic diets was similar to that of commercial feedstuffs used in shrimp culture (P>0.05). The *in vitro* digestibility was still lower than the values reported for desired growth in ponds (1g, Lemos *et al*., 2008, Viera *et al*., 2022)

Further studies should validate the *in vitro* results through animal growth in the breeding ponds.

CONCLUSIONS

The pH-stat method showed *in vitro* increases in diet digestibility by combining beer barley husks and industrial shrimp residues, thus indicating that alternative feeds may have similar digestibility to commercial ones.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: JOLO, AAG, AAC; data analysis and interpretation: JOLO, AAG, AAC; redaction of the manuscript: JOLO, AAG, AAC.

CONFLICT OF INTEREST STATEMENT

The authors declare the existence of no conflicts of interests.