**Application**

The significant interaction between feed and pH highlights the complexity of digestive processes and underscores the importance of considering multiple factors when evaluating feed quality.

**Introduction**

In vitro digestibility methods have become increasingly important in evaluating the nutritional quality of feed ingredients and diets for various species, including aquaculture (Moyano et al., 2015). These techniques aim to simulate the digestive processes that occur in the gastrointestinal tract, providing valuable insights into nutrient availability and digestibility without the need for extensive animal trials. However, the results obtained from the in vitro model maybe varies due to many factors including incubation pH. The goal of the study is to determine the effect of pH and feed substrate on nitrogen protein digestibility.

**Material and methods**

A 6 × 2 full factorial design was used in this study to evaluate the effects of incubation pH (1.36 vs. 3.13, representing the gut environments of salmonids and tilapia, respectively) and feed substrates (soybean meal, whey powder, black soldier fly larvae powder, waxworms, faba beans, and bioethanol yeast). Each substrate was tested in triplicate for each pH level.

The protein solubility of the feed was determined according to Araba and Dale (1990). In brief, 2 g of each dry feed substrate (in triplicate) and 0.08 g of pepsin were weighed into 15 mL tubes. Subsequently, 10 mL of HCl (0.0001 HCl for pH 3.13 samples and 0.1 HCl for pH 1.36 samples) was added to each tube. The tubes were vortexed then incubated in a water bath at 28°C for 18 hours and 24 minutes. After incubation, the samples were vortexed again, centrifuged at 4500 rpm for 5 minutes, and the supernatant was collected. Diluted samples were prepared by mixing 100 µL of supernatant with 900 µL of distilled water. From this, 25 µL of the diluted sample was pipetted into the wells of a microplate then, 200 µL of the working reagent (prepared using the Pierce™ BCA Protein Assay Kit) was added to each well. The microplate was incubated at 37°C for 30 minutes and subsequently analysed using a plate reader (Thermo Scientific Multiskan FC) at 620 nm against a standard curve. A two-way analysis of variance was performed, and means were compared using the Fisher’s LSD test. Data of the study was analysed using R 4.4.2.

**Table 1.** Effect of feed and incubation pH on in vitro protein digestibility of fish.

|  |  |  |
| --- | --- | --- |
|  | Protein solubility (μg soluble protein/g DM) | |
| Feed | pH1=1.36 | pH 2=3.13 |
| Bioethanol yeast | 544c | 420d |
| Black soldier fly larvae powder | 602bc | 540cd |
| Faba beans | 699ab | 557c |
| Soyabean meal | 531c | 472cd |
| Waxworms | 691cabc | 724b |
| Whey powder | 743a | 878a |
|  |  |  |
| SEM | 45.2 |  |
| P |  |  |
| Feed | <0.001 |  |
| pH | 0.112 |  |
| Feed\*pH | 0.04 |  |

pH: incubation pH. SEM: standard error mean. Means within a column with different superscripts are significantly different (p≤ 0.05).

**Results**

There were significant effects of feed (p<0.001), and the interaction between feed and incubation pH (P= 0.04) on protein solubility. At pH 1.36, faba beans had significantly higher protein solubility compared to soybean meal. However, these feeds did not significantly differ in protein solubility at pH 3.13.

**Conclusion**

The effect of protein source on in vitro protein solubility was influenced by incubation pH. Although significant differences in protein solubility were observed across feeds, it is important to note that these variations may not directly correspond to differences in fish performance.

**Acknowledgments**

Nottingham Trent University poultry research unit team is acknowledged for feed analysis.

**References**

Araba, A., and Dale, N. 1990. Poultry Science, 69, 76-83.

Moyano, F.J., Sáenz de Rodrigáñez, M.A., Díaz, M., and Tacon, A.G.J. 2015. Reviews in Aquaculture, 7(4), 223-242.