Pepsin-Pancreatin Digestibility Of Various Protein Sources Intended For Pet Food

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Introduction

Pet food companies need quality protein ingredients. Several ingredients have been proposed as potential protein sources in pet food but lack information. Evaluating these proteins by animal feeding tests are costly in time and resources. An in vitro (benchtop) method could provide detailed information about the protein quality for novel ingredients that would be fast and efficient. Limited nutritional information regarding the digestibility of crude protein in potential pet food ingredients has constrained the product portfolio for many companies. By developing a pepsin-pancreatin digestibility assay, protein digestibility for certain ingredients can be determined and allow these ingredients to be considered for pet food applications in the marketplace.

Objectives

- To develop a pepsin-pancreatin digestibility assay and determine the appropriate incubation time for enzyme hydrolysis.

Materials & Methods

- Ingredients evaluated were faba beans, navy beans, spray dried granulated egg, chickpeas, pea protein isolate (72% CP) and pea protein concentrate (50%CP), green field peas, and sunflower meal.
- 15 ml of 0.1N HCl-pepsin solution was produced and then added to 1 g of the sample ingredient. This was then incubated for 3 or 6 hours at 37°C.
- After pepsin incubation, 7.5 ml of 0.5N NaOH was added to each tube. This neutralized the sample and stopped the hydrolysis of pepsin.
- A mixture of 4mg of pancreatin pH8 phosphate buffer, and 1 ml of sodium azide was added to the test tube. This initiated pancreatin digestion and prevented microbial growth. Test tubes were then incubated for 18 hours at 37°C.
- Samples were centrifuged (20,000 x G), washed, filtered, and dried at 105 °C overnight.
- Residual protein was determined with a modified Kjheldal assay.
- Data was analyzed with statistical software using the GLIMMIX procedure for mixed models (SAS v 9.4, SAS institute Inc, Cary, NC).

Creating the Reagents

HCl-Pepsin solution was created by adding 1.5mg of pepsin to every 1ml 0.1N HCL

0.2M Phosphate buffer pH8 was created by adding 26.9g of sodium phosphate dibasic anhydrous and 1.9g of sodium phosphate monobasic anhydrous to 700 mL distilled water. Then completed to volume with pH adjusted

Figure 1. Enzyme preparations developed to determine protein digestibility of proteins

Figure 2. Flowchart of protein digestion of foodstuff

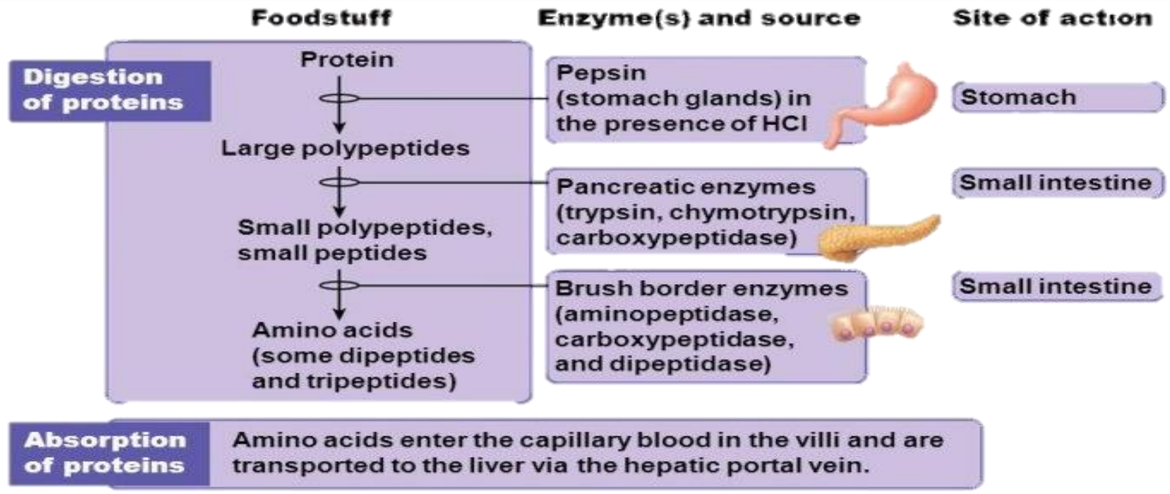

Figure 2. Flowchart of protein digestion of foodstuff (Pearson Education, 2015)

Results

Table 1. Effect of time on protein digestibility at 3 and 6 hours of pepsin incubation time[1]

Ingredient, %	Crude Protein %	3 hr Digestibility	6 hr Digestibility
Ground Chick Pea	24.50	98.51^{ab}	98.62^{a}
Ground Faba Bean	33.50	99.23^{a}	98.86^{a}
Ground Green Pea	25.20	99.01^{a}	99.06^{a}
Ground Navy Bean	23.30	94.81^{bc}	92.87^{c}
Pea Protein 50%	55.50	98.58^{ab}	96.39^{abc}
Pea Protein 72%	79.80	85.03^{d}	84.48^{d}
Spray Dried Granulated Egg	53.30	99.50^{a}	99.48^{a}
Sunflower Meal	40.70	83.68^{d}	86.55^{d}

$^{a-d}$Values differ p<0.05, ^{1}Diet p<0.001; Time p=0.69; Diet*Mix=0.63

Conclusions

The crude protein digestibility differed among the diets tested with spray dried granulated egg the highest (p<0.05). Incubation time did not affect the crude protein digestibility of the protein sources.

Based on these results, the crude protein digestibility was greater than expected, which would suggest other factors in the procedure should be altered in order to attain a protein digestibility estimate consistent with reported animal evaluations.

References

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