# Development of a laboratory technique to simulate pig feed digestion

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In recent years, the high cost of feed in pig production has increased the economic utilization of high fibrous feedstuffs as an alternative to high cost cereals feedstuffs (Le Goff *et al*., 2002). These fibrous feedstuffs such as brewer's and milling by-products are used as total or partial replacement of energy source ingredients for pig diets. While there is increased interest in evaluating the nutrients present in the fibrous feedstuffs, *in vivo* method, a direct measurement of feed digestibility has been reported costly, time and labour consuming and ethically difficult (Boisen & Fernandez, 1997). However, the results of *in vitro* (laboratory measurement) digestibility of pig diets has been found to be highly correlated to the *in vivo* (direct measurement in the animal) digestibility estimates (Boisen & Fernandez, 1997; Akinsola, 2013). (*In vitro* means in glass)

This research study became a paradigm shift investigation of record that has adapted tube method to nylon bag technology for the determination of dry matter and energy digestibility of fibrous feeds for pigs. The *in vitro* experiment was carried out by simulating the environment of the stomach, small and the large intestines as closely as possible under strictly controlled conditions.

## Materials and methods

Feed samples were milled (1 mm) separately, oven-dried to a constant weight in a Labotec® drought oven and cooled in a desiccator. Ninety six Ankom® F57 filter bags were pre-rinsed in pure (99% and colourless) acetone and completely air-dried. An aliquot of 0.5 g of each sample was subsequently weighed into prepared weighed bags and afterwards heat sealed with an impulse sealer. The weight of the filter bag plus sample was recorded as initial weight prior digestion.

Figure 1 Ankom Daisy$^{II}$ incubator and fermentation jar with bags containing feed samples

Figure 2: A pig fitted with an ileal canula standing in a metabolism crate in a conventional *in vivo* digestibility study

Twenty four samples of six different formulated fibrous diets (soya bean-maize, soya beans hulls, wheat, brewer spent grain, lucerne and maize cob based diets) differing in their chemical composition were placed in each of the four 4L incubation jars (24 samples x 4 digestion jars = 96 samples / run). To each of the jars were added phosphate buffer and hydrochloric acid (HCl) solutions and the pH adjusted to 2.0 using HCl or sodium hydroxide (NaOH) solution. The jars were then placed on a rotating rack in the incubator. Heat and agitation switches for warming and intermittent rotation of the jar respectively were tuned on and the temperature equilibrated to 39 $^{o}$C. The jars were taken out, pepsin and chloramphenicol solution (to inhibit fermentation) were added to the digestion medium and subsequently digested for 2 h using the built in timer. This digestion step was to simulate digestion in the pig stomach.

Jars were afterwards taken out and the pH was adjusted to 6.8 in order to simulate the environment of the small intestine. Pancreatin was then added to each jar and digested for another 5 h. At the completion of 5 h digestion period, sample bags were taken out, rinsed thoroughly with warm water and subsequently rinsed with ethanol and acetone respectively. Sample bags were then oven-dried and the weight loss at the end of the first two enzymatic digestions was used to estimate ileal digestion. Disappearance of nutrients (DM and energy) was regarded as being digested *in vitro.*

Sample residues from the ileal digestion were further digested with Viscozyme, a fibrolytic enzyme cocktail for 24 h in a freshly prepared buffer with pH of 4.8. This digestion step was to simulate fibre degradation in pigs. At the completion of the fibre degradation process, jars were taken out and rinsing and drying procedures for ileal digestion was followed. Bags with residues were later weighed and recorded as the final weight for each sample to calculate total tract (or faecal) digestibility. Disappearance of nutrients was also regarded as being digested *in vitro*

**Results**

The results of *in vitro* (digestion in the Ankom Daisy$^{II®}$ incubator) dry matter and energy digestibility of six fibrous pig diets were validated *in vivo* (digestion in live pigs) using the same diets. The results of *in vitro* and *in vivo* digestibility methods were compared and are depicted in Table 1.

**Table 1; Mean faecal values (% ± standard deviation) and equations of regression lines for *in vitro* and *in vivo* dry matter and energy digestibility of the experimental diets**

| | Faecal DM digestibility (%) | | Energy digestibility (dE) (%) | |
|---|---|---|---|---|
| Diet | *In vitro* | *In vivo* | *In vitro* | *In vivo* |
| Control* | 91.10 | 86.81 | 90.48 | 86.51 |
| Maize cob | 85.99 | 80.68 | 85.17 | 80.50 |
| Lucerne | 86.85 | 82.32 | 85.54 | 81.83 |
| Brewers spent grains | 80.54 | 75.54 | 78.96 | 74.36 |
| Wheat bran | 84.88 | 82.35 | 83.53 | 81.42 |
| Soya bean hulls | 89.20 | 84.74 | 88.27 | 84.26 |
| Regression equation (Model)[1] | y=0.9426x | | y=0.955x | |
| Adjusted $R^2$ | 0.9446 | | 0.9364 | |

*Soya bean-maize based diet
[1] Linear model for y = bx, where y = *in vivo* digestibility estimate, b = slope, x = *in vitro* digestibility estimate

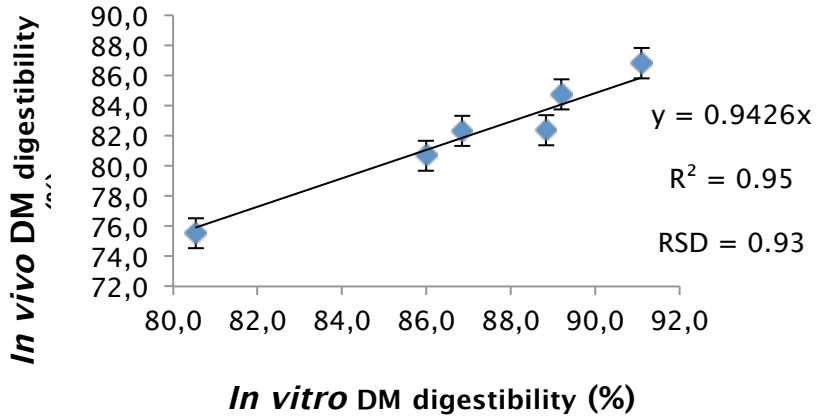


**Figure 1: The relationship between *in vivo* and *in vitro* dry matter digestibility estimates**

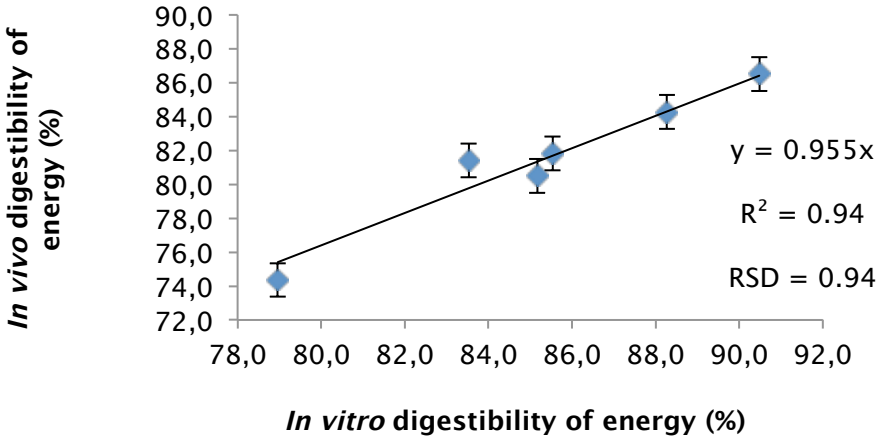

**Figure 2: The relationship between *in vivo* and *in vitro* energy digestibility estimates**

## Discussion

The use of the Ankom Daisy$^{II}$® incubator for prediction of feedstuff and diet digestibility estimates has been identified as easy, cheap, and labour efficient analysis when compared to the conventional tube method (Akinsola, 2013). The results of this study showed a closed linear relationship between *in vitro* and *in vivo* digestibility estimates for DM (y = 94x; $R^2$ = 0.95) and energy (y = 95x; $R^2$= 0.94) respectively. The equations showed that *in vitro* values were multiplied by 0.94 and 0.95 respectively to obtain the predicted *in vivo* values for DM and energy. The results also indicated that the developed *in vitro* technique can be used to estimate apparent total tract of DM and energy of common feedstuffs and diets for pigs with aa 94% accuracy. Further work is needed to also determine whether amino acid digestibility can be predicted with sufficient accuracy.

## References

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