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Comparative Evaluation of in Vitro and in Vivo Nutrient Digestibility of Dietary Levels of Rice Husk Supplemented With or Without Commercial Enzyme

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ABSTRACT

The effects of supplemental enzyme on *in vitro* and *in vivo* digestibility of nutrients in rice husk were determined in a 2 x 4 factorial combinations. Rice husk was added at 0 or 25% inclusion level at the expense of maize in the control diet. Each of these was undertaken in the presence of no enzyme (0ppm) or with different types of commercial enzyme at recommended level, which are Roxazyme G, (150ppm), Nutrase xyla, (100ppm) and Phytase, (100ppm). Thus there were 8 treatments for the *in vivo* trial, each with 3 replicate cages of eight chicks. One hundred and ninety-two (192) samples were used for the *in vitro* experiment under a given procedure to mimic the artificial digestive system, one hundred and ninety-two (192) day old unsexed arbor acres broilers were used for the *in vivo* experiment. Generally it was observed in this study that increase in the content of rice husk in the absence of supplementary enzymes either in the *in vitro* or *in vivo* trials caused a decrease in the nutrient digestibility particularly for protein and fibre ($P<0.05$) . However, there was improved nutrient digestibility of diets supplemented with enzymes in this study for both the *in vivo* or *in vitro* trials. There was a good correlation between the *in vitro* determinations and the *in vivo* results; hence, conclusively there should be an *in vitro* trial prior the actual *in vivo* introduction.

Keywords: *in vivo*, *in vitro*, rice husk, digestibility, commercial enzymes

INTRODUCTION

Efforts to extract more nutrients from conventional and non- conventional feedstuffs have been a focus for research for decades. In recent times more effort has been directed towards harnessing and utilizing by-products and wastes which are not directly utilizable by man, and take advantage of convertible mechanism of animal organ to convert what is seen as a waste into wholesome animal product for human consumption with the use of enzymes. Peter and Hoffman (2002) reported enzyme as a practical tool offering the possibility of replacing the expensive raw material with cheaper ones. Evaluation of nutritive value of feeds can be done in various ways which include the use of *in vitro* and *in vivo* techniques, *in vivo* means "within the living" and refers to experimentation done in or on the living organism while *in vitro* means "within the glass" and refers to the technique of performing a given procedure in a controlled environment outside a living organism. When carrying out *in vivo* digestibility, the diet under investigation is given to the animal in known amount and the output of faeces measured. More than one animal are used, because animals of the same species, age and sex differ slightly in their digestive ability (Mcdonald et al, 1987). However, the strength of *in vitro* model experiments is that trials can be repeated under exactly the same conditions in a series of experiments. It is widely recognized that *in vivo* animal experiments are the ultimate test to determine the actual effects of feed enzymes. However there is a dearth of information on the *in vitro* study. Hence the need to evaluate comparatively the *in vitro* and *in vivo* digestibility of rice husk supplemented with or without enzyme in order to have a tool that allow a complete *in vivo* prediction from an *in vitro* studies.

MATERIALS AND METHODS

In the *in vivo* model- one hundred and ninety-two (192) day old mixed sex broilers of a commercial strain were used for this experiment. The birds were housed in an electrically heated battery cage and

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were fed the experimental diet shown in Table 1, from day old to 8 weeks of age. The experimental diet consisted of a 2 × 4 factorial combination of replacement of maize with rice husk and commercial enzymes. Rice husk was added at 0 or 25% inclusion level at the expense of maize in the control diet. Each of these was undertaken in the presence of no enzyme (0ppm) or with different types of commercial enzyme at recommended level, which are Roxazyme G, (150ppm), Nutrase xyla, (100ppm) and Phytase, (100ppm). Thus there were 8 treatments, each with 3 replicate cages of 8 chicks.

Table1. *Composition of Experimental Diets*

Ingredients	1	2	3	4	5	6	7	8
Basal*	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Rice husk	_	_	_	_	25.00	25.00	25.00	25.00
Maize	50.00	50.00	50.00	50.00	25.00	25.00	25.00	25.00
Roxazyme G	_	150ppm	_	_	_	150ppm	_	_
Nutrase	_	_	100ppm	_	_	_	100ppm	_
Phytase	_	_	_	100ppm	_	_	_	100ppm
Analyzed nutrient content								
Dry matter								
Crude protein (%)	88.21	89.45	89.86	89.63	86.75	87.65	87.72	87.92
Crude fat (%)	18.42	19.46	19.56	19.91	19.65	20.65	20.70	20.77
Crude fibre (%)	6.05	5.95	5.90	5.75	10.05	10.10	10.07	10.12

**Made up of groundnut cake 26.80%, blood meal 3.00% palm kernel cake 3.00%, fish meal 2%, maize milling waste 10%, bone meal 2.35%, oyster shell 0.25%, salt 0.25%, palm oil 2.00%, Di-methonine 0.10%, premix 0.25 (provid ed per kg; Vit. A 4,000IU;Vit. D3 8000IU; Vit. E 4,000mg, Vit. K 900mg, Vit. B1 500mg; Vit. B2 2000mg; Vit B3 5,500mg; Choline chloride 15,000mg; Antioxidant (BHT)0.05%; Iron 1.8%; copper 0.2%;manganese 2.4%; cobalt 0.045%; zinc 2.8%; iodine 0.04%; selenium 0.18%; calcium 12.8%).*

- Treatment not included

The *in vitro* trial - the experimental sample consisted of a 2 × 4 factorial combination as shown in Table 1; there were eight treatments each with twenty-four replicate. Thus one hundred and ninety-two (192) samples were used for this experiment also. Four grams of each sample was weighed into a test tube, 10mls of pepsin in 0.1M hydrochloric acid was added and the content incubated at 40^{o}C for 30minutes on a shaker, samples were then neutralized with 0.2M sodium hydroxide followed by addition of 5 mls of pancreatin (0.2%w/v) in a buffer solution, such that a pH of 6 – 7 was maintained. The mixture was incubated for 2 hours with shaking at a temperature of 40^{o}C. Thereafter, the content of each test tube was filtered using weighed filtered paper and a vacuum pump. The filtrate (digested material) was discarded while the residue was oven-dried at 70^{o}C for 24hours. Residues were then weighed and subjected to proximate analysis.

Nutrient Digestibility

The *in vivo* trial - A nutrient digestibility trial was undertaken when the birds were 3weeks old. Weighed quantities of feed were supplied and excreta collected over a 72hours. The excreta samples were weighed, oven dried at 70^{o}C and weighed again to determine their dry matter. Dried excreta were ground prior to chemical analyses. Nutrient digestibility was calculated using the formula below for the *in vivo* trial

$$ND = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}} \times 100$$

ND = Nutrient digestibility

Nutrient intake = weight of dry feed intake × coefficient of nutrient in feed

Nutrient output = weight of dry excreta × coefficient of nutrient in faeces

Nutrient digestibility was calculated using the formula below for the *in vitro* trial,

$$ND = \frac{\text{NS} - \text{NR}}{\text{NS}} \times 100$$

NS = Nutrient in sample (4g of feed × coefficient of nutrient in feed)

NR = nutrient in residue (weight of residue × coefficient of nutrient in residue)

Proximate Analysis

The diets and samples of oven-dried residues were subjected to proximate analysis using the method of the A.O.A.C. (1995). Crude protein was determined using the kjeldahl procedure. Ether extract was determined by subjecting the samples to petroleum ether (b.p.60-80^{o}C) extraction in a soxhlet apparatus. Crude fibre of the samples was determined by the method described by Cullison (1982).

Statistical Analysis

All the data were subjected to Analysis of Variance using the model for factorial design and the significant differences between means were compared using Duncan's Multiple Range Test (1955).

RESULTS AND DISCUSSION

Table 2 showed the effects of the dietary treatment on *in vivo* nutrient digestibility. There was no significant effect of the treatment on fat digestibility ($p > 0.05$). However, there were significant interaction between dietary level of rice husk and the type of enzyme on protein and crude fibre digestibility ($p<0.05$). Thus, increase in the dietary level of rice husk in the absence of supplemented enzyme caused a significant decrease in protein digestibility. There were significant variations in fibre digestibility with or without enzyme. In the absence of enzyme supplementation fibre digestibility reduced with increase in dietary level of rice husk. The experiment showed that birds fed the diets supplemented with enzymes were more efficient in protein and fibre digestibility compared to those fed diets without enzyme supplementation.

Table2. *in vivo nutrient digestibility of dietary levels of rice husk supplemented with or without enzyme.*

Dietary treatment	Protein (%)	Fat (%)	Fibre (%)
Level of rice husk (A)	*	NS	*
0	64.80^{b}	84.05	58.50^{b}
25	63.42^{a}	84.00	53.70^{a}
Enzyme treatment (B)	*	NS	*
No enzyme 0ppm	63.81^{a}	83.62	52.50^{a}
Roxazyme(150ppm)	67.30^{b}	83.20	57.00^{b}
Nutrase (100ppm)	67.50^{b}	83.20	57.05^{b}
Phytase (100ppm)	68.00^{b}	83.25	56.92^{b}
A X B	*	NS	*
SEM	1.50	0.92	1.56

NS: Non – Significant

** Means within the same column followed by different superscripts are significantly different (P<0.05).*

Table 3 shows that fibre and protein digestibility in the artificial digestive system decreased with increase in the inclusion level of rice husk without enzyme supplementation, however increase in the inclusion level of rice husk supplemented with enzymes resulted in higher fibre and protein digestibility. More so, there was no significant effect of the treatment on fat digestibility as shown in Table 3.

Table3. *In vitro nutrient digestibility of dietary levels of rice husk supplemented with or without enzyme.*

Dietary treatment	Protein (%)	Fat (%)	Fibre (%)
Level of rice husk (A)	*	NS	*
0	64.30^{b}	84.30	57.40^{b}
25	63.10^{a}	84.10	55.40^{a}
Enzyme treatment (B)	*	NS	*
No enzyme 0ppm	63.00^{a}	85.50	52.10^{a}
Roxazyme G (150ppm)	66.20^{b}	85.70	58.10^{b}
Nutrase (100ppm)	65.93^{b}	85.60	58.40^{b}
Phytase (100ppm)	66.07^{b}	85.62	57.90^{b}
A X B	*	NS	*
SEM	1.42	0.91	1.46

NS: Non – Significant

** Means within the same column followed by different superscripts are significantly different (P<0.05)*

Table 4 shows that there were significant correlations of the in vitro and in vivo results on the nutrient digestibility. Cereal based diets contain non starch polysaccharide (NSP) that reduced the utilization of nutrient. Arabinoxylans are the main NSP that increase viscosity of digestive content, hence interfered with digestion, which can be detrimental to nutrient utilization. Enzymes are known to decrease viscosity of digestive content resulting in improvement in nutrient digestibility (Bedford and Classen, 1993). Generally it was observed in this study that increase in the content of rice husk in the absence of supplementary enzymes either in the *in vitro* or *in vivo* models caused a decrease in the nutrient digestibility particularly for protein and fibre, the reduction in nutrient digestibility is associated with the fibre that has high level of NSP. Mod et al, (1982) opined that the release of nutrient from fibrous feedstuff with addition of exogenous enzyme is possible *in vitro*. It is well documented that enzyme supplementation to diets increase digestibility of nutrients (Koregay 2001; Atteh 2000; Lee *et al*, 2003, Selle *et al*, 2007 and Akinwumi *et al*, 2012), thus there was improved nutrient digestibility of diets supplemented with enzymes in this study either in the *in vivo* or *in vitro* models. The *in vitro* determinations were similar to the *in vivo* results which agree with Cardot *et al*, (2007) who reported that in vivo results can be predicted based on *in vitro* data.

Table4. *Comparison of the in vivo and in vitro results on protein and fibre digestibility.*

Nutrient	Mean	Standard deviation	Correlation co-efficient
Crude protein (%)			
In vitro	65.64	5.20	
In vivo	68.54	5.25	0.97**
Crude fibre (%)			
In vitro	58.72	5.24	
In vivo	55.47	5.33	0.88**

** Correlation is significant at the 0.01 level (2-tailed).*

CONCLUSION

There was a good correlation between the *in vitro* determinations and the *in vivo* results; hence, conclusively there should be an *in vitro* trial prior the actual *in vivo* introduction in order to have a tool that allows a complete *in vivo* prediction from an *in vitro* study.

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