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Use of *In vitro* Technique in Predicting *In vivo* Response of Poultry to Enzyme Supplementation

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Authors' contributions

This work was carried out in collaboration between both authors. Author AJO designed this study. Author JA performed In vitro and In vivo trials as well as laboratory and statistical analysis. Author JA also wrote the protocol and the first draft of the manuscript. Author AJO managed the analyses of the study while author JA managed the literature searches. Both authors read and approved the final manuscript

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ABSTRACT

The need for rapid test in evaluation of feedstuffs in poultry nutrition cannot be overemphasized. Such test must however be able to replace exactly the response in the animal concern. This study was conducted to determine the suitability of *In vitro* technique in predicting the *In vivo* response of poultry to enzyme supplemented feedstuffs. Rice husk was used in a Completely Randomized Design with individual and cocktail of enzymes for both *In vitro* and *In vivo* trials. Three exogenous enzymes namely a xylanase, a multipurpose and a phytase were used individually, pairwise and altogether with the feedstuff to constitute the treatments namely T1, T2, T3, T4, T5, T6, T7, and T8. Each treatment was replicated thrice for both *In vitro* and *In vivo* trials. The *In vitro* trial was done to simulate the chicken digestive system while the *In vivo* trial was done using the intubation method. Parameters measured for both trials were digestibility values for dry matter, crude protein, ether extract, crude fiber and fiber fractions. All data collected were statistically analyzed using Analysis of Variance procedure and treatment means were separated using Duncan Multiple Range Test. Correlation analysis was carried out to compare the results of both trials. Results show that enzymes individually and as cocktails significantly improved the digestibility of parameters for both *In vitro* and *In vivo* trials. Cocktails of enzymes were significantly better than

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the individual enzymes for dry matter and crude fiber digestibility for both *In vitro* and *In vivo* trials. Correlation analysis shows positive correlation (r=0.99, r=0.96, r=0.94, r= 0.86, r=0.78) between *In vitro* and *In vivo* trials in most of digestibility values for the rice husk. It was concluded that *In vitro* trial can be used as both criterion and replacement for *In vivo* trial when determining the efficacy of exogenous enzymes in poultry nutrition.

Keywords: Cocktail; correlation; enzymes; intubation; in vitro; in vivo.

1. INTRODUCTION

Digestibility experiments are very useful in estimating the feeding value of novel feedstuffs in animal nutrition. As a nutritive value index, digestibility values provide a biologically meaningful parameter that can be used in routine feed evaluation [1]. Feeding trials are mostly conducted to estimate these values like digestibility coefficient for the nutrients or the utilization of additives. However, feeding trials are characterized by long time and high cost [2].Thus cheaper and quicker methods are essential to predict/determine the feeding value of novel feedstuffs before being fed to live animals. These methods include prediction of digestibility from chemical composition and *In vitro* [1]. The use of chemical composition involves determining feeding value through calculation. This method is simple and reasonably rapid as it involves only determination of proximate composition. It's also relatively cheap. However, it does not account for the ability of the animal to digest components like lignin and cellulose [1]. This is because these variables (lignin and cellulose) considerably alter digestibility of a feed with little, if any, effect on its chemical composition. There is therefore the need for rapid feed evaluation technique because of the practical limitation of the commonly used approaches like table values, prediction equations [1]. These limitations include cost, logistics, time etc., making them less practical to be applied in routine feed evaluation by industry [1]. These have necessitated the development of other methods like *In vitro* digestion, fermentation and Near Infra-Red technology which are getting more attention by Nutritionists and feed industry for their capability to evaluate feedstuffs relatively more quickly and more cost-effective [1]. The use of *In vitro* method involves simulating the *In vivo* condition in a laboratory environment. *In vitro* methods have the advantage of not only being less expensive and less time-consuming, but they allow maintaining experimental conditions more precisely than do *In vivo* trials [1].

Exogenous enzymes are of different profile and different activities. Thus, it may be impossible for one enzyme to achieve complete breakdown of crude fibre and other complex components of the feed stuff [3]. This has led to the proposal for the addition of several enzymes on the same feedstuff to see whether this will improve the digestibility of the feed stuff beyond the effect of the individual enzyme. This phenomenon is known as enzyme cocktail and is still a subject of research. According to [4] cocktails of enzymes performed significantly better than individual enzymes in their effects on *In vitro* digestibility of rice husk.

Rice husk is the outermost covering of the paddy grain. It is the hard protective covering of the grain during growing season. It is obtained during milling of the paddy rice and it is about 20% of the paddy weight [5]. It is very low in nutritional value for both polygastric and monogastric animal. This is because of its high fibre content and high lignin content [5]. Attempts have been made to enhance the utilization in poultry nutrition. [6] reported that ensiling rice husk with 5% molasses for 21 days made it suitable for effective utilization by broilers considering the growth performance, hematological indices and cost analyses. It has been observed by [4] that exogenous enzymes individually and as cocktails improved *in vitro* degradability of crude protein, ether extract and crude fiber of rice husk compared to the control. Therefore, the use of exogenous enzymes holds potential for the improved utilization of this feedstuff.

This study was conducted to investigate the correlation between *In vitro* and *In vivo* trials in determining the efficacy of enzyme cocktails on rice husk in poultry nutrition. The hypothesis for the study was to test whether *In vitro* trial can be used as a replacement for *In vivo* trial in the evaluation of enzyme supplemented feedstuffs.

2. MATERIALS AND METHODS

2.1 Experimental Design

The *In vitro* and *In vivo* experiments were conducted simultaneously and the results

compared. Completely randomized design was used in each trial as shown in Table 1. Three different exogenous enzymes were used for the study and they were a xylanase (a bacterial endo-xylanase), a multipurpose enzyme (fungal enzyme containing xylanase, glucanase, hemicellulase and cellulase) and a phytase. They were included at manufacturers' recommended inclusion level of 100ppm for xylanase, 150 ppm for multipurpose enzyme and 150ppm for phytase.

The enzymes were used individually and as cocktails of two and three enzymes as shown in Table 1. For Cocktails the enzymes were included at ratio of 100 ppm: 150 ppm: 150 ppm (Xylanase: multipurpose: phytase). Each treatment was replicated thrice giving a total of twenty four experimental units. This design was used for both *In vitro* and *In vivo* trials. The xylanase used in this study has 9000 units of xylanase activity per gram as stated by the manufacturer. It is produced from *Bacillus substilis* and it is powdery in nature and cream colored. The enzyme complex has wheat flour as its carrier and the recommended inclusion level is 100ppm.

The multipurpose enzyme used is produced from *Trichoderma viride*. It is a granular and odorless solid preparation. The enzyme complex has 5-10% active enzyme and the manufacturer's recommended inclusion level is 150ppm. It has 26,000 units/gram of endo 1,4,-β-xylanase , 18000 units/g of endo 1,3,[4]-glucanase, 8000 units/g of endo 1,4 β-glucanase, 8000 units/gram of cellulase and traces of pectinase, hemicellulase, α-amylase and others as stated by the manufacturer.

The phytase used is 3-phytase enzyme obtained from *Aspergillus nige*r. It is granular in nature and it has activity of 5000FTU/gram as stated by the manufacturer. One FTU (phytase unit) is the amount of enzyme which liberates 1 micromole (1 µmol) of inorganic phosphate per minute from sodium phytate at pH of 5.5 and temperature of 37^{o}C

2.2 *In vitro* Trial

In vitro digestion trial was carried out in line with the procedure of [7] with some modifications. The two-step digestion procedure simulated the chicken's gastric and intestinal digestions. Rice husk was obtained from a commercial feed mill in Ilorin, North central Nigeria. It was ground into mash form. The proximate composition and fiber fractions were determined. Rice husk was milled to pass through 1.00 mm sieve and used to prepare each treatment with the respective enzyme or cocktail (Table 1). One kilogram of each treatment was prepared and five gram was put in a 50ml flask and 10ml of pepsin in 0.1M HCl (aq) was added. The content was incubated for 30 minutes at temperature of 40^{0}C and P^{H} of 2.0. It was then neutralized with 0.2M NaOH and 10ml of pancreatin in a buffer solution was added and incubated for additional 2 hours at temperature of 40^{0}C and p^{H} of 7.0. The two stages of the incubation were accompanied with shaking with the aid of a mechanical shaker. At the end of the digestion stages, the content of the flask was filtered using a weighed filter paper. The filtrate was discarded whereas the residue was prepared for determination of proximate composition and fibre fractions using the procedures of [8] and [9] respectively.

The *In vitro* digestion took place at the Central Research Laboratory of the University of Ilorin while proximate analyses and fibre partitioning were done at the Department of Animal Production Laboratory, University of Ilorin, North central Nigeria. The *In vitro* trial lasted for three hours.

Table 1. Composition of experimental treatments

Test material	Treatments							
	T1	T2	T3	T4	T5	T6	T7	T8
Rice husk (%)	100	100	100	100	100	100	100	100
Xy[1] (ppm)	--	100	--	--	100	100	--	100
Mp[2](ppm)	--	--	150	--	150	--	150	150
Ph[3](ppm)	--	--	--	150	--	150	150	150

1: Xylanase enzyme 2: Multipurpose enzyme 3. Phytase enzyme

T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase

2.3 *In vivo* Trial

Twenty four adult black cockerels of approximately equal weight (about 2.2 Kg) were used. They were randomly allocated to the battery cage with one bird in a cell representing a replicate. There were eight treatments and three replicates per treatment. The birds were provided with feed and water *ad libitum* before the commencement of experiment.

The *In vivo* trial was done using the intubation method as described by [10] with some modifications. All the birds were deprived of feed for 21 hours prior to the administration of the treatment so as to empty the digestive system. At exactly 21hours, a cockerel was removed from its cell and a tube of about 8mm internal diameter was inserted into the crop of the cockerel via the oesophagus. A Plastic funnel was placed on top of the tube. Sixty grams of the treatment prepared (rice husk plus each enzyme or cocktail as shown in Table 1) in form of mash was placed in the funnel and gently pushed down with the aid of a glass rod. Water was then added to rinse the feedstuff off the funnel and the tube. After this procedure the fed bird was returned to the cell and this procedure was repeated for each of the birds. The time for the intubation for each bird was recorded. Immediately after the feeding for each bird, feacal collection tray was placed under the individual cell and feacal samples were collected over a period of 24 hours after the intubation from all the cockerels. Adequate water was provided for all the birds prior to and after the intubation. At exactly 24hours after placement, the feacal collection tray was removed from each of the cells. The feacal sample was collected and prepared for chemical analysis of interest.

2.4 Laboratory Analysis and Calculations

Proximate analysis and fiber partitioning were carried out for the feacal samples using the procedure of [8] and [9] respectively. The following calculations were made after the trials.

Nutrients degradability values for *In vitro* trial were calculated using the formula below:

$$\text{Nutrient Degradability (\%)} = \frac{\text{Nutrient in sample (g)} - \text{Nutrient in Residue (g)}}{\text{Nutrient in Sample (g)}} \times 100$$

Apparent Nutrient digestibility value for *In vivo* trial was calculated using the formula below:

$$\text{Apparent Nutrient Digestibility (\%)} = \frac{\text{Nutrient in Treatment (g)} - \text{Nutrient in Feaces (g)}}{\text{Nutrient in Treatment (g)}} \times 100$$

2.5 Statistical Analyses

Values of *In vitro* nutrients degradability and *In vivo* nutrients digestibility obtained were statistically analyzed using one way ANOVA procedure of [11]. Significant differences between treatments' means were determined using [12]. Correlation analysis was carried out to compare the values from both *In vitro* and *In vivo* trials using the Statistical Analysis Software [11].

3. RESULTS

3.1 Effects of Enzymes on *in vitro* Degradability of Rice Husk

Table 2 shows the effects of the treatments on *In vitro* degradability of rice husk. The dry matter degradability was not significantly different ($P<0.05$) among treatments T8 (cocktail of the three enzymes), T5 (cocktail of xylanase and multipurpose enzymes) and T7 (cocktail of multipurpose and phytase enzymes). All the enzymes individually and as cocktails significantly ($P<0.05$) improved the dry matter degradability and crude fibre degradability of rice husk compared to the control. Among the three enzymes used individually, the multipurpose enzyme (T3) performed significantly best ($P<0.05$) on crude fibre digestibility followed by xylanase (T2) and phytase (T4) which were also significantly different. Treatments T3 (multipurpose enzyme) and T8 (cocktail of the three enzymes) were significantly higher ($P=.05$) than the control (T1) in their effects on crude protein degradability while treatments T4 (phytase), T6 (cocktail of xylanase and phytase) and T7 (cocktail of multipurpose and phytase) were significantly lower than the control in their effects on crude protein degradability. There were significant differences ($P=.05$) across the treatments in their effects on *In vitro* degradability of ether extract of rice husk. There were significant differences ($P=.05$) between treatments T6 (cocktail of xylanase and phytase) and T8 (cocktail of the three enzymes) in their effects on ether extract degradability. All the cocktails (T5, T6, T7 and T8) significantly ($P=.05$) improved degradability of ether extract compared to their individual enzymes.

Table 3 shows the effects of the treatments on *In vitro* degradability of fibre fractions of rice husk.

The treatments were significantly different (*P*=.05) in their effects on degradability of neutral detergent fibre, cellulose and hemicellulose. All the enzymes individually (T2, T3 and T4) and as cocktails (T5, T6, T7 and T8) significantly (*P*=.05) improved the degradability of neutral detergent fibre, cellulose and hemicellulose. The effects of treatments T3 (multipurpose enzyme) and T7 (cocktail of multipurpose enzyme and phytase) on degradability of acid detergent fibre (ADF) were not significantly different .However, there were significant differences (*P*=.05) in the effects of other treatments on degradability of ADF and all the enzymes improved ADF degradability significantly compared to the control. All the experimental treatments (T2 to T8) had no significant effects on degradability of acid detergent lignin (ADL) compared to the control (T1).

3.2 Effects of Enzymes on *in vivo* Digestibility of Rice Husk

Table 4 shows the effects of the enzymes and their cocktails on *In vivo* digestibility of rice husk. All the experimental treatments significantly (*P*=.05) improved the dry matter digestibility of rice husk compared to the control. The effects of treatments T8 (cocktail of the three enzymes), T5 (cocktail of xylanase and multipurpose enzymes) and T7 (cocktail of multipurpose and phytase enzymes) on dry matter digestibility were not significantly different (*P*=.05). The effects of treatments T2 (xylanase) and T4 (phytase) were not significantly different (*P*=.05) from each other but were significantly different from T3 (multipurpose enzyme) which had the significantly (*P*<0.05) highest effect among the three enzymes (47.65%).

All enzymes, with the exception of phytase (T4), significantly (P<0.05) improved the digestibility of crude fibre compared to the control. There were significant differences between the effects of the individual enzymes on crude fibre digestibility and multipurpose enzyme (T3) gave the significantly (*P*=.05) highest effect of 28.63%. There was no significant difference between cocktail of xylanase and multipurpose enzymes (T5) and cocktail of the three enzymes (T8) in their effects on crude fibre digestibility. There were no significant differences between the cocktails (T5, T6, T7 and T8) in their effects on digestibility of ether extract. However, all enzymes and their cocktails with the exception of phytase enzyme (T4) significantly (*P*=.05) improved the digestibility of ether extract compared with control.

All the enzymes individually and as cocktails significantly (*P*=.05) improved crude protein digestibility of rice husk compared to the control while there were no significant differences (*P*=.05) between the treatments (cocktails) T5, T7 and T8 on crude protein digestibility. However treatment T6 (cocktail of xylanase and phytase enzymes) was significantly different (*P*=.05) from the other cocktails. There was no significant difference between treatments T2 (xylanase) and T4 (phytase) in their effects on crude protein digestibility but their effects were significantly lower (*P*=.05) than the effect of treatment T3 (multipurpose enzyme).

3.3 Effects of Enzymes on *in vivo* Digestibility of Fibre Fractions of Rice Husk

All the enzymes individually and as cocktails significantly (*P*=.05) improved the digestibility of neutral detergent fibre (NDF) compared to the control (Table 5). There were significant differences (*P*=.05) between the individual enzymes (T2, T3 and T4) on NDF digestibility and the multipurpose enzyme (T3) gave the significantly (*P*=.05) highest effect whereas phytase gave the least effect with 51.46% and 9.82% respectively as shown in Table 5. Cocktail of the three enzymes (T8) gave the significantly (*P*=.05) highest effect of 68.92% on digestibility of NDF compared to the other cocktails whereas cocktail of xylanase and phytase (T6) gave the least effect. All the enzymes and their cocktails improved the digestibility of acid detergent fibre (ADF) compared to the control. There was no significant difference between treatments T5 (cocktail of xylanase and multipurpose enzymes), treatment T7 (cocktail of multipurpose enzyme and phytase) and treatment T8 (cocktail of the three enzymes) in their effects on ADF digestibility. Among the three enzymes individually, the multipurpose enzyme (T3) had the significantly (*P*=.05) highest effect on digestibility of ADF. All the enzymes individually and as cocktails significantly (*P*=.05) improved digestibility of cellulose compared to the control. Treatments T2 (Xylanase) and T4 (phytase) were not significantly different **(***P*=.05**)** in their effects on digestibility of cellulose and were significantly lower (*P*=.05) than the effect of treatment of T3 (multipurpose enzyme). All the treatments were not significantly different (*P*=.05) from each other

in their effects on digestibility of acid detergent lignin (ADL).

3.4 Correlation Analysis between *In vitro* and *In vivo* Trials

Correlation analysis for digestibility values for *In vitro* and *In vivo* experiments(Table 6) revealed near perfect relationship for hemicellulose (r =0.94, *P*=.05), cellulose (r = 0.86, *P*=.05), Acid Detergent Fibre (r = 0.96, *P*=.05), Neutral Detergent Fibre (r = 0.99, *P*=.05) and crude fibre (r = 0.94, *P*=.05) (Table 6). Linear positive relationship exist for Dry Matter (r = 0.78, *P*=.05) and crude protein (r = 0.10, *P*=.05).There was however negative correlation between *In vitro* and *In vivo* digestibility values for Acid Detergent Lignin (r = -0.08) and ether extract (r = -0.02).

Table 2. Effects of enzymes on proximate composition of rice husk using *in vitro* technique

	Treatments								
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**SEM**
DM (%)	20.04^{d}	39.54^{c}	41.30^{b}	38.78^{c}	45.43^{a}	41.74^{b}	45.35^{a}	46.66^{a}	1.66
CF (%)	0.34^{g}	32.71^{e}	40.80^{d}	7.22^{f}	57.28^{b}	40.28^{d}	47.16^{c}	67.93^{a}	4.51
EE (%)	64.44^{d}	61.78^{h}	63.05^{e}	62.31^{g}	66.32^{b}	62.94^{f}	65.93^{c}	67.52^{a}	0.43
CP (%)	51.00^{c}	51.09^{c}	51.97^{a}	50.49^{d}	50.89^{c}	50.61^{d}	50.54^{d}	51.28^{b}	0.13

a, b, c, d, e, f, g: means in the same row followed by the same superscript are not significantly different (P=.05) DM=Dry matter, CF=Crude fibre, EE=Ether extract, CP=Crude protein, T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase

Table 3. Effects of enzymes on fibre fractions of rice husk using *in vitro* technique

	Treatments								
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**SEM**
NDF (%)	0.50^{h}	26.64^{f}	52.79^{d}	11.98^{g}	66.13^{b}	37.88^{e}	60.31^{c}	75.15^{a}	5.22
ADF (%)	0.02^{g}	20.92^{e}	45.67^{c}	9.67^{f}	55.88^{b}	27.69^{d}	47.35^{c}	62.11^{a}	4.39
CELL. (%)	0.09^{g}	15.02^{e}	44.78^{c}	4.85^{f}	50.28^{b}	34.32^{d}	52.47^{b}	61.43^{a}	4.56
HEMI. (%)	0.45^{h}	25.33^{f}	47.10^{d}	11.14^{g}	59.21^{b}	33.92^{e}	50.54^{c}	63.34^{a}	4.44
ADL (%)	0.01^{a}	0.01^{a}	0.00^{a}	0.01^{a}	0.01^{a}	0.01^{a}	0.01^{a}	0.01^{a}	0.01

a, b, c, d, e, f, g, h: means in the same row followed by the same superscript are not significantly different. (P=.05)

NDF=Neutral detergent fibre, ADF=Acid detergent fibre, CELL.=Cellulose, HEMI.=Hemicellulose, ADL=Acid detergent lignin, T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase.

Table 4. Effects of enzymes on *in vivo* digestibility of rice husk

	Treatments								
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**SEM**
DM (%)	30.07^{f}	35.04^{e}	47.65^{c}	35.98^{e}	52.28^{b}	39.25^{d}	50.48^{b}	56.19^{a}	2.66
CF (%)	19.80^{f}	22.60^{e}	28.63^{cd}	19.90^{f}	33.40^{ab}	24.50^{de}	30.08^{bc}	35.30^{a}	4.39
EE (%)	44.50^{c}	46.70^{b}	47.07^{ab}	45.60^{bc}	49.06^{a}	47.05^{ab}	47.25^{ab}	48.16^{a}	1.28
CP (%)	41.07^{e}	45.80^{d}	53.13^{b}	47.60^{cd}	59.61^{a}	49.14^{c}	59.29^{a}	61.29^{a}	3.36

a, b, c, d, e, f, g: means in the same row with same superscript are not significantly different (P=.05) DM=Dry matter, CF=Crude fibre, EE=Ether extract, CP=Crude protein, T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase

Table 5. Effects of enzymes on *in vivo* digestibility of fibre fractions of rice husk

	Treatments								
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**SEM**
NDF (%)	0.99^{g}	25.21^{e}	51.46^{c}	9.82^{f}	59.99^{b}	41.27^{d}	55.88^{c}	68.92^{a}	4.86
ADF (%)	0.66^{e}	13.70^{d}	40.57^{b}	10.79^{e}	55.66^{a}	24.56^{c}	53.76^{a}	59.18^{a}	4.57
CELL. (%)	1.76^{f}	6.18^{e}	21.42^{c}	3.47^{e}	41.28^{b}	11.34^{d}	22.31^{c}	49.10^{a}	3.55
HEMI. (%)	5.02^{f}	20.41^{e}	36.03^{d}	7.31^{a}	47.24^{b}	17.34^{e}	40.14^{c}	54.69^{a}	3.76
ADL (%)	0.03^{a}	0.03^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.00

a, b, c, d, e, f, g: means in the same row with same superscript are not significantly different (P=.05)
NDF=Neutral detergent fibre, ADF=Acid detergent fibre, CELL.=Cellulose, HEMI.=Hemicellulose, ADL=Acid detergent lignin, T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase

Table 6. Correlation analysis between *in vitro* and *in vivo* digestibility values for rice husk using enzymes

	DMIVT	**CPIVT**	**CFIVT**	**EEIVT**	**NDFIVT**	**ADFIVT**	**ADLIVT**	**CELLIVT**	**HEMIIVT**
DMIV	.78**								
CPIV		.10							
CFIV			.94**						
EEIV				-.02					
NDFIV					.99**				
ADFIV						.96**			
ADLIV							-.08		
CELLIV								.86**	
HEMIIV									.94**

*** Correlation is significant at the 0.01 level (2-tailed)*
DMIVT=In vitro Dry matter, CPIVT=In vitro Crude protein, CFIVT=In vitro crude fibre, EEIVT=In vitro ether extract, NDFIVT=In vitro neutral detergent fibre, ADFIVT=In vitro acid detergent fibre, ADLIVT=In vitro acid detergent lignin, CELLIVT=In vitro cellulose, HEMIIVT=In vitro hemicellulose, DMIV=In vivo Dry matter, CPIV=In vivo Crude protein, CFIV=In vivo crude fibre, EEIV=In vivo ether extract, NDFIV=In vivo neutral detergent fibre, ADFIV=In vivo acid detergent fibre, ADLIV=In vivo acid detergent lignin, CELLIV=In vivo cellulose, HEMIIV=In vivo hemicellulose

4. DISCUSSION

Rice husk is an agricultural by-product of rice milling and is available in abundance in rice growing parts of Nigeria where it constitutes a nuisance to the environment [13]. Traditionally, rice husk has been used as feedstuff for ruminants but it has little or no nutritive value for poultry [14]. This is due largely to its high fibre content as well as its phytate level and in most rice mills it is left to rot or used as fuel. Rice husk is highly fibrous with about 44 % crude fibre (of which over 90% is insoluble fibre) and 56.2 % cellulose [4]. It also has 15.2% ADL and low crude protein (3%). According to [15], attempts at feeding rice husk to poultry resulted in poor growth performance as a result of low nutritional quality, high fibre and lignin content. [16] reported that weight gain values significantly decreased (P=.05) as the level of rice husk increased in the diet of pullet chicks and it was recommended that inclusion of grit at 5% in the diets of chicks containing rice husk can improve nutrient retention and reduce feed cost. Attempts have been made to improve utilization of rice husk by chemical or microbiological modification. These modifications reduce the dietary fibre and increase the available carbohydrates.

According to [17], an increase in the dietary level of rice husk without commercial enzyme supplementation significantly (P=.05) decreased nutrient digestibility and weight gain of broiler birds. However birds fed rice husk diets supplemented with commercial enzymes performed better in all parameters tested than those fed rice husk diets without commercial enzyme. Monogastric animals including poultry are able to utilize rice husk as a feed ingredient better when additives like grits or enzymes are added [16]. According to [18], biodegradation of rice husk with *Trichoderma viride* resulted in

improvement in the energy content of the treated samples when compared to the untreated in addition to improvement in proximate composition. Significant reduction in the cell wall content of the rice husk was observed as the period of degradation increases. Results of the *In vitro* trial in this study also buttressed the aforementioned authority showing that with the presence of exogenous enzymes, there was improvement in the digestibility of rice husk. The effects of fibre and phytate on digestibility of other nutrients like protein, ether extract and carbohydrate has been well documented [19]. Thus, it could be inferred that improvement in the degradation of these substances (phytate and fibre) is expected to elicit improvement in the digestibility of the affected nutrients. This can be noticed in the *In vitro* and *In vivo* trials although more prominent in the *In vivo* where increase in crude fibre digestibility correspond with increase in digestibility of ether extract and crude protein.

Developing a rapid digestibility assay for novel feedstuffs and feed additives is essential for many reasons. The *In vitro* digestibility trial can be of importance in this regard. *In vitro* method simulates the activity of the gastro intestinal tract of the animals to determine the digestibility of nutrient [7]. The *In vitro* digestion techniques can also be used to screen large set of samples in a relatively short period of time and is non-invasive to animals and relatively very cheaper than *In vivo* methods [1]. According to [20], one of the tools for predicting *In vivo* results based on *In vitro* data is good correlation. For *In vitro* digestibility values to be of relevance for predicting *In vivo* response of animals there must be positive correlation between the two values. The positive correlation coefficients obtained in this study for most of the parameters indicate the relevance of *In vitro* results to *In vivo* results. In the opinion of [1], it is imperative that any simulation experiment (*In vitro*) should correlate well with *In vivo* parameters before such *In vitro* results could replace the *In vivo* in prediction. In this study, most parameters have liner relationship ranging from r = 0.78 to r = 0.99 and this indicates the suitability of *In vitro* technique in predicting the *In vivo* response of poultry species to enzyme supplementation. This finding is in agreement with that of [21]. However, there was negative correlation for ether extract and Acid detergent lignin with r= -0.02 and -0.08 respectively. Acid detergent lignin is generally not digestible. The recalcitrance of lignin is as a result of unique lignocellulose structure formed by a chemical polymerization of three aromatic monomers namely coumaryl, coniferyl and sinapyl to form heterogenous three dimensional polymer that lacks chirality [22].

According to [23], degradation of cell wall Non Starch Polysaccharides by both xylanases and glucanases is the main contributing factor to the greater digestion and absorption of nutrients. It was observed by [24] that cellulase supplementation significantly improved the digestibility of cell wall components. Thus the improved digestibility attributed to the multipurpose enzyme in the digestibility of rice husk in this study may be due to the presence of cellulase, xylanase and glucanase. This has made it better than the single purpose Xylanase with only xylanase activity. According to [25], increasing fibre concentration of feed causes decreased digestibility of all nutrients, reduced weight and increased feacal bulk. Fibre act as a diluent agent which lowers nutrient concentration and the effect of this indigestible fraction of carbohydrates can be observed on the anatomy of the digestive tract, the transit time, water losses bringing about poor digestion in monogastric animals [26]. However, results of this experiment show improvements not only in digestibility of crude fibre but also that of crude protein and ether extract compared to the control. Enzyme supplementations efficiently break down the arabinoxylans in the feed stuff, thereby resulting in a decrease in intestinal viscosity and consequent improvement in digestibility of nutrients [16]. Results obtained in this study are similar to that of [17] where *grindazyme* (multipurpose enzyme) performed significantly best among the trio of Xylanase, Phytase and *grindazyme*.

5. CONCLUSION

The correlation coefficients between the *In vivo* and *In vitro* digestibility values for most of the parameters in this study were positive and high. This implies a good relationship between the *In vitro* and *In vivo* trials. Hence, there should be an *In vitro* trial prior to the actual *In vivo* investigation in order to have a tool that justifies the *In vivo* investigation where necessary. It is hereby concluded that *In vitro* digestibility method can be used as both a criterion and a substitute for the estimation of *In vivo* apparent digestibility of enzyme-supplemented feedstuffs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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