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Efficacy of supplementary enzymes on *in vitro* digestibility of various ratios of maize with wheat offal

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ABSTRACT

The basic experimental diets were 4 x 5 factorial combinations of the inclusion levels of wheat offal at the expense of maize in the control diet with different enzymes. Basically the control diet had 100% of maize while in other diets 25, 50, 75 or 100% of maize was replaced with wheat offal. Each of these was replicated thrice and undertaken in the presence of no enzyme, with different types of enzymes at the recommended level or a combination of different types of enzymes. Thus were a total of 60 treatment samples and then the *in vitro* digestibility trial was carried out. The results showed that increase in the level of wheat offal at the expense of maize without enzyme supplementation caused significant decrease in nutrient digestibility ($P < 0.05$). However, inclusion of fungal or bacterial enzyme and their combinations improved nutrient digestibilities *in vitro* ($P < 0.05$).

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1. Introduction

Availability and price of maize are influenced by competition between man, industry and livestock; hence it makes economic sense to find cheap alternatives for maize in poultry diet. Research has shown that it is possible to partially or completely replace maize with cheap agro-industrial waste (Ogbonna *et al*, 1993). Efforts to extract more nutrients from feedstuffs both conventional and non-conventional have been a focus for research for

decades. In recent times more efforts has been directed toward *in vivo* digestibility trial for these alternative feed ingredients. However the *in vitro* digestibility techniques offers one potential method for evaluation of nutritive value of feed, *in vitro* systems are also valuable tools to investigate the mode of enzyme action and the development of new feed enzyme application (Morten *et al*,2004). The *in vitro* digestibility techniques is known to avoid the utilization of animals, hence the strength of *in vitro* model experiments is that trials can be repeated under exactly the same conditions in a series of experiments while *in vivo* studies are subject to the large physiological developmental stages and then animals of the same species, age and sex differs slightly in their digestibility ability (McDonald *et al*, 1987).

There is some information on the *in vivo* investigation on enzyme supplementation of feed ingredients of poultry. However the efficacy of *in vitro* enzyme supplementation on the digestibility of high fibre feed ingredients has not been fully addressed. Hence, this study aimed at investigating *in vitro* digestibility of diets based on substitution of wheat offal for maize with or without enzyme addition.

2. Materials and methods

The basic experimental diets were 4 * 5 factorial combinations of the inclusion levels of wheat offal at the expense of maize in the control diet with different enzymes. Basically the control diet had 100% of maize while in other diets 25, 50, 75 or 100% of maize was replaced with wheat offal as shown in table 1. Each of these was replicated thrice and undertaken in the presence of no enzyme, with different types of enzymes at the recommended level or a combination of different types of enzymes. Thus were a total of 60 treatment samples.

Table 1

Composition of experimental diets

Items	Diets																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Maize	100	75	50	25	0	100	75	50	25	0	100	75	50	25	0	100	75	50	25	0
Wheat offal	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
No Enzyme	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fungal Enzyme	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Bacterial Enzyme	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
Combination of Enzymes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+

- Treatment not included +Treatment included

2.1. The *in vitro* digestibility experiment

Four grams of each diet was weighed in three replicates into a test tube, 10mls of pepsin in 0.1M hydrochloric acid was added and the content incubated at 40°C for 30 minutes on a shaker, Samples were then neutralized with 0.2M sodium hydroxide followed by addition of 5mls 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°C. Thereafter, the content of each test tube was filtered using weighed filter paper and a vacuum pump. The filtrate (digested material) was discarded while the residue was oven-dried at 70°C for 24 hours.

2.2. Analysis

The diets and samples of oven-dried residues were subjected to proximate analysis (Table 2) using the method of the AOAC (2008) Crude protein was determined using the Kjeldahl procedure. Ether extract was determined by subjecting the samples to petroleum ether (b.p 60-80°C) extraction in a soxhlet apparatus. Crude fibre of the samples was determined by method described by cullison (1982). 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).

Table 2

Analyzed nutrient content of diet based on substitution of wheat offal for maize.

Analyzed content	Diet 1	Diet 2	Diet 3	Diet 4
Dry Matter (%)	89.20	95.27	94.00	90.70
Crude Protein (%)	8.75	12.68	14.22	16.40
Crude Fat (%)	3.80	3.50	3.40	3.00
Crude Fibre (%)	2.50	6.30	10.00	11.30
ME(MJ/Kg)	14.31	7.58	6.08	5.22

3. Results and discussion

Table 3 showed the effects of ratios of maize to wheat offal and types of enzymes on *in vitro* nutrient digestibility. Increase in dietary level of wheat offal in absence of enzyme caused a significant decrease in protein, fat and fibre digestibilities. However, supplementing diet with fungal enzyme caused a significant increase ($P < 0.05$) in protein and fibre digestibilities. Protein digestibility for diets supplemented with bacterial or a combination of enzymes were comparable ($P > 0.05$) irrespective of the ratio of wheat offal. Crude fat showed significant decrease ($P < 0.05$) with increasing levels of wheat offal. However, there was significant increase in digestibility with enzyme supplementation. Fibre digestibility above ratio 1: 1 inclusion level of wheat offal without enzyme supplementation showed a significant decrease in fibre digestibility.

Table 3Effects of ratio of maize to wheat offal and types of enzymes on *in vitro* nutrient digestibility.

Level of wheat offal (LW)	Crude protein (%)	Crude fat (%)	Crude fibre (%)
Ratio of Maize : Wheat Offal	*	*	*
4:0	63.40 ^c	70.30 ^c	55.40 ^c
3:1	62.30 ^c	69.90 ^c	53.70 ^b
1:1	61.50 ^c	67.10 ^b	53.60 ^b
1:3	55.10 ^b	66.70 ^b	43.80 ^a
0:4	52.50 ^a	65.30 ^a	41.50 ^a
Type of Enzyme (TE)	*	*	*
No Enzyme	64.30 ^a	67.30 ^a	55.50 ^a
Fungal Enzyme	69.10 ^c	71.60 ^b	58.80 ^c
Bacterial Enzyme	65.70 ^b	70.40 ^b	56.30 ^b
Combination of Enzymes	65.30 ^b	76.40 ^c	55.50 ^a
LW x TE	*	*	*
SEM	2.76	2.08	0.77

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

There are many reasons for exclusion of high fibrous feed ingredient in poultry diets, among which is decreased digestibility of nutrient (Bach, 1997) as a result of arabinoxylans which are the main Non Starch Polysaccharide (NSP) contained in cereal based feed ingredients. Arabinoxylans are known to increase viscosity of digestive content, hence interfered with digestion which can be detrimental to nutrient utilization. Enzymes help to decrease viscosity of digestive content resulting in improvement in nutrient digestibility. Mod *et al* (1983) opined that the release of nutrient from fibrous feedstuff with addition of exogenous enzyme is possible *in vitro*. In this study, increase in the level of wheat offal in the absence of supplementary enzyme caused a decrease in nutrient digestibility while the addition of supplementary enzyme improved nutrient digestibility. This observation agrees with the results of Cowieson and Acamovic (2003) when peanut was supplemented with enzymes. Furthermore, supplementation of the diets in this study with combination of enzymes had better effect on crude fat this might be as a result of synergistic effects of different enzymes on the diet. According to Creswell (1994) enzyme cocktail should be more effective and better to those supplemented with individual enzyme. Results of this study showed that fungal enzyme was efficient in fibre digestion which is in agreement with Akinfemi and Mukar (2012) who reported improved digestibility of lignin with fungal treatment. The increase

digestibility of fibre could be apparently related to fungal ability to unlock polysaccharide molecules in the diet breaking down the long chains into smaller units (Jozefiat *et al*, 2004). The significant interaction ($P < 0.05$) between dietary level of wheat offal and types of enzymes, Table 4 showed that bacterial enzyme supplementation generally increased nutrient digestibility when ratio of maize to wheat offal is not more than 1:1. With combination of maize: wheat offal above 1:1 decreased nutrients digestibilities was observed. This observation suggest that bacterial enzyme used in this trial may not be able to digest efficiently at ratios more than 1 : 1 of maize to wheat offal.

4. Conclusion

Generally it was observed that most of the results obtained in this study on the *in vitro* digestibility trial were similar to previous *in vivo* digestibility trials on high fibre content feed ingredient supplemented with enzymes. It is therefore concluded that an *in vitro* determinations could be conducted first to predict the digestibility of feed supplemented with enzyme before the *in vivo* introduction. Furthermore based on the results of this study the maize; wheat offal combination supplemented with fungal enzyme will be recommended.

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