Scientific Research and Essay Vol. 4 (6) pp. 555-559, June, 2009
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2009 Academic Journals

Full Length Research Paper

Enzyme cocktail for enhancing poultry utilisation of cocoa pod husk

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Accepted 23 March, 2009

Cocoa Pod Husk (CPH) is a major by-product from the cocoa industry in Ghana. Using CPH as a low-cost unconventional feedstuff is hampered by its poor nutrient composition due to high level of non-starch polysaccharides including cellulose, pectin and hemicellulose, which are indigestible in monogastric livestock such as poultry. An *in vitro* enzyme treatment study was conducted to test the effect of various combinations of selected exogenous fibrolytic enzymes on the digestibility of CPH feedstuff. Concentrations of 0.8, 0.6 and 0.8% w/w respectively for Pentopan®MonoBG, Viscozyme®L and Pectinex®5XL were observed as appropriate levels for supplementing CPH feedstuff. Among the enzyme combinations tested, the Pentopan®MonoBG + Viscozyme®L, Viscozyme®L + Pectinex®5XL and Pentopan®MonoBG + Viscozyme®L + Pectinex®5XL formulae were most effective in maximising sugar release from CPH feedstuff by 42 - 53% increase with a corresponding reduction (7 - 14%) in crude fibre and non-starch polysaccharide fractions ($P < 0.05$). The present results suggest that supplementation with multi-enzymes or blends of exogenous NSP-degrading enzymes may enhance the capacity of poultry to efficiently digest and utilise dietary CPH.

Key words: Agro-industrial by-product, cocoa pod husk, non-starch polysaccharides, *in vitro* treatment, fibrolytic enzymes, saccharification.

INTRODUCTION

With current rising costs of conventional feed ingredients, animal nutritionists have advocated for the use of agro-industrial by-products as unconventional feed stuff, which are cheaper and available in large quantities in countries with agro-based economies. Cocoa Pod Husk (CPH) which forms over 70% (w/w) of the whole matured fruit of Cocoa (*Theobroma cacao* L.), is a major agro-industrial by-product from the cocoa industry.

Ghana is currently the world's second largest producer of cocoa after La Cote d'Ivoire with annual production level of over 600,000 metric tonnes (ICCO, 2003) and thus, at every cocoa harvesting season, enormous quantities of CPH become available but are currently under-utilised. Previous works revealed the potential use of CPH as an unconventional low-cost feed ingredient for livestock nutrition, possibly reducing feed costs by replacing some of the expensive conventional feed ingredients used in ration formulation (Atuahene et al., 1984; Sobamiwa, 1998). However, the inclusion of CPH in diets for monogastric livestock is challenged by its poor nutrient composition.

It is highly fibrous with significant amounts of cell wall components including lignin (14% w/w) and non-starch polysaccharides (NSPs) mainly hemicellulose (11%, w/w); cellulose (35%, w/w) and pectin (6%, w/w).

Monogastrics such as poultry and pigs do not produce enzymes to utilise these polysaccharides, which increase

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gut viscosity with a resultant adverse effect on animal growth and performance (Annison, 1990; Sobamiwa, 1993). Enzyme supplementation is well documented as effective in breaking polymeric chains of NSPs and hence improving the nutritive value of fibrous feedstuffs (Elmakeel et al., 2007; Giraldo et al., 2008).

Due to the chemical structure of plant cell wall matrix, a combination of various fibrolytic enzyme activities (including cellulases and hemicellulases) has been recommended to enhance saccharification of NSPs (Beldman et al., 1987). To date, literature information on enzyme treatment of CPH feed stuff is not available. Thus this present *in vitro* study was conducted to screen various blends of selected NSP-degrading commercial enzymes and to suggest a suitable enzyme mixture for supplementing CPH to enhance its utilisation in poultry nutrition.

MATERIALS AND METHODS

Preparation of cocoa pod husk and enzymes used

Fresh cocoa pod husks were obtained and processed at Cocoa Research Institute of Ghana (CRIG), New Tafo-Akim. The husks were cleaned, chopped into smaller pieces and solar-dried to about 9-10% moisture content. The dried husk pieces were then ground with a hammer mill to produce the CPH meal (1 mm average particle size) and stored in polyethylene bags until needed for the study. Commercial enzyme preparations from *Aspergillus* species investigated were Pentopan®MonoBG (endo-1,4-xylanase activity with declared activity of 2500 FXU w/g), Viscozyme®L (beta endo-1,3(4)-glucanase activity with declared activity of 100 FBG/g as well as side activities of xylanase, cellulase and hemicellulase) and Pectinex®5XL (polygalacturonase activity with declared activity of 4500 PECTU/ml as well as arabinase side activity).

These enzymes were chosen because of their orientation to break bonds known to limit utilisation of carbohydrates particularly NSPs present in CPH. All enzyme preparations were obtained from Novozymes North America Inc., USA.

In vitro enzyme treatment of CPH

The study employed the Bedford and Classen (1993) two-stage *in vitro* digestion assay, without using pepsin and pancreatin. This digestion procedure is a simple and rapid screening technique and has been useful for determining the efficacy of feed enzymes as well as establishing their best effective dosage levels. By varying the enzyme concentrations (0.0 - 2.5%, w/w), the minimum dosage levels of the individual enzymes were initially determined prior to their combination and administration as enzymes mixtures for the CPH treatment.

One gram of the ground CPH was transferred into 50 ml centrifuge tube and then incubated with the enzyme preparation at concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2 and 2.5% w/w, plus 30 ml of 0.1 N HCl at 40°C with occasional vortexing to simulate the pH environment at the peptic or gastric phase. After 3 h, 10 ml of 1 M $NaHCO_3$ solution was added and incubated at 40°C for 6 h with occasional vortexing to simulate the pH condition at the pancreatic or intestinal phase. At the end of the incubation the contents were filtered using Whatman No. 1 paper. The filtrate was assayed for total sugars or soluble carbohydrates released while the residue was dried and analysed for crude fibre content. All determinations were replicated.

Treatment of CPH with enzyme mixtures

Different enzyme combinations, namely Pentopan®MonoBG + Viscozyme®L (PMBG + VL); Pentopan®MonoBG + Pectinex®5XL (PMBG + PXL); Viscozyme®L + Pectinex® 5XL (VL + PXL); Pentopan®MonoBG + Viscozyme®L + Pectinex®5XL (PMBG + VL + PXL), at their minimum individual dosages earlier established, were used to treat CPH. The Bedford and Classen (1993) digestion assay outlined above was followed for each treatment. At the end of the incubation period, filtrates obtained were assayed for total sugars released, whiles the fibrous insoluble residues ('raffinate') were analysed for the level of fibre fractions including NDF, ADF, lignin-sa, cellulose and hemicellulose. All determinations were replicated.

Chemical analyses

Proximate components of CPH were determined by using AOAC (1990) procedures: ash (method 942.05), protein (method 954.01) and fibre (method 962.09). The Anthrone-H_2SO_4 reaction method International Livestock Research Institute ILRI (1997) was used to measure total sugars or soluble carbohydrates.

Fibre fractions including NDF (assayed with a heat stable amylase); ADF and lignin-sa (lignin determined by solubilization of cellulose with sulphuric acid) were determined according to procedures as described by Mertens (2002), AOAC (1990)-method 973.18, and Robertson and Van Soest (1981), respectively. Hemicellulose content was estimated by difference between NDF and ADF values and the difference between ADF and lignin-sa values represented cellulose content.

Statistical management of data

Variance between treatments was analyzed using GENSTAT Release 7.2 DE statistical software for PC/Windows XP (2007). When the analysis of variance revealed the existence of significant differences among the treatment means at the 5% level, the Fisher's (protected) Least Significant Difference (LSD) was used to locate treatment means that were significantly different from one another.

RESULTS AND DISCUSSION

CPH Fibre composition

Table 1 shows the proximate composition of CPH and it reveals the lignocellulosic nature of CPH due to the high levels of fractions including lignin and non-starch polysaccharides. However, the measured values differ from those previously reported by Sobamiwa (1993) and Osei et al. (1991). A large proportion of the differences could be attributed to cocoa variety or species from which the CPH was obtained as well as other agronomic factors including characteristics of soil for cultivation and harvesting time (Sobamiwa and Longe, 1993).

Table 1. Proximate composition of cocoa pod husk.

Components	g/kg DM
Dry Matter	889.6 ± 1.5
Total Ash	90.7 ± 0.4
Crude protein	91.4 ± 1.7
Crude fibre	357.4 ± 0.9
NDF	597.8 ± 18.8
ADF	470.4 ± 9.3
Lignin	211.6 ± 2.6
Hemicellulose	127.5 ± 9.6
Cellulose	261.5 ± 3.0
Total Sugars	33.0 ± 0.6

Values are presented based on dry weight material as Mean ± Standard deviation.
DM = Dry Matter.

Minimum enzyme dosages for CPH saccharification

Figure 1 shows the change in total sugars released from CPH at varying treatment concentrations (0.2 - 2.5%, w/w) of the individual enzymes. Generally, significant rise in total sugars was observed as enzyme level increased from 0.2 to 2.5% (w/w). At a least concentration of 0.8% (w/w), both PMBG and PXL treatments resulted in optimal release in total sugars by 12.72 and 19.24% respecttively ($P < 0.05$).

On the other hand, VL treatment effect was optimum at 0.6% (w/w), giving a 33.94% increase in total sugars ($P < 0.05$). These increases in total sugars were accompanied by corresponding significant reduction in crude fibre; 3.36, 7.35 and 5.57% respectively, by PMBG, VL and PXL at their minimum dosages (Figure 1).

Variation in enzyme activities contributed significantly to the amount of sugars released. PMBG exhibits a major xylanase activity which degrades xylan polymers. Xylan is a major fraction of hemicelluloses in plant cell walls (Bhat and Hazlewood, 2001). The higher percent increase in total sugars observed in the VL treatment may be probably due to the multi-activity feature it exhibits. Apart from its major cellulose-degrading activity, side activities of xylanase and hemicellulase are also present in VL, thus offering a broad spectrum of degrading activity on NSPs of CPH including cellulose and hemicellulose.

Effect of combined enzymes on CPH saccharification

Table 2 shows the effect of different combinations of the commercial enzymes on sugar release (saccharification) as well as levels of fibre fractions of CPH. Total sugars released following the combined enzyme treatments ranged from 47.1 g to 50.7 g per kg CPH. Percent increase in total sugars was least (36.97%) for the PMBG + PXL treatment but highest (53.64%) when the three enzymes were combined (PMBG + VL + PXL).

However, the VL + PXL and PMBG + VL + PXL treatments produced similar increases in total sugars ($P > 0.05$). Increase in total sugars also resulted in a corresponding decline in the levels of crude fibre, non-starch polysaccharides, NDF and ADF (Table 2). Among the various combined enzyme treatments studied, the PMBG + VL + PXL treatment resulted in maximum reduction ($P < 0.05$) in the crude fibre, NDF and ADF levels by 14.07, 14.05 and 11.10% respectively with similar effect observed in the VL + PXL treatment ($P > 0.05$). However, the effects of PMBG + VL and VL + PXL treatments on CPH were only similar ($P > 0.05$) in terms of total sugars released and crude fibre. Lignin content of CPH was generally unaffected ($P > 0.05$) by any of the enzyme treatments.

The observed enhanced saccharification of CPH polysaccharides by the combined enzymes can be primarily attributed to a synergy between the activities of individual enzymes in the various enzyme mixtures. Enzyme synergism has been demonstrated in previous works including Brenes et al. (1993) who used α-galactosidases with other enzymes to improve the nutritional value of raw lupin seeds.

In addition, Sreenath et al. (1999) reported a significant degree of synergism of enzymes in the saccharification of untreated alfalfa fibre, when they observed maximum release of sugars from alfalfa fibre following treatment with combined mixture of commercial cellulase and pectinase, compared to treatment with the individual enzymes.

Fibre fractions: NDF (mainly made up of hemicellulose, cellulose and lignin) and ADF (consist of cellulose and lignin) are often negatively correlated with dry matter intake and animal digestibility of forage feedstuff respecttively (Van Soest et al., 1991). Performance of mono-gastrics compared to ruminants is adversely affected by high dietary fibre, because in addition to their endogenous enzyme action, ruminants have ruminal microorganisms that augment their capacity to generate extra useful energy from dietary fibre through fermentation.

Thus, the observed appreciable reduction in NSP fractions of CPH due to the hydrolytic effect of the test enzyme enzyme mixtures in this study could be regarded as positive from the standpoint of improved feed quality or digestibility, especially for poultry and pigs. The absence of ligninolytic activity in any of the enzyme preparations is largely responsible for the insignificant change in lignin level of CPH.

Lignin is a complex non-carbohydrate aromatic polymer

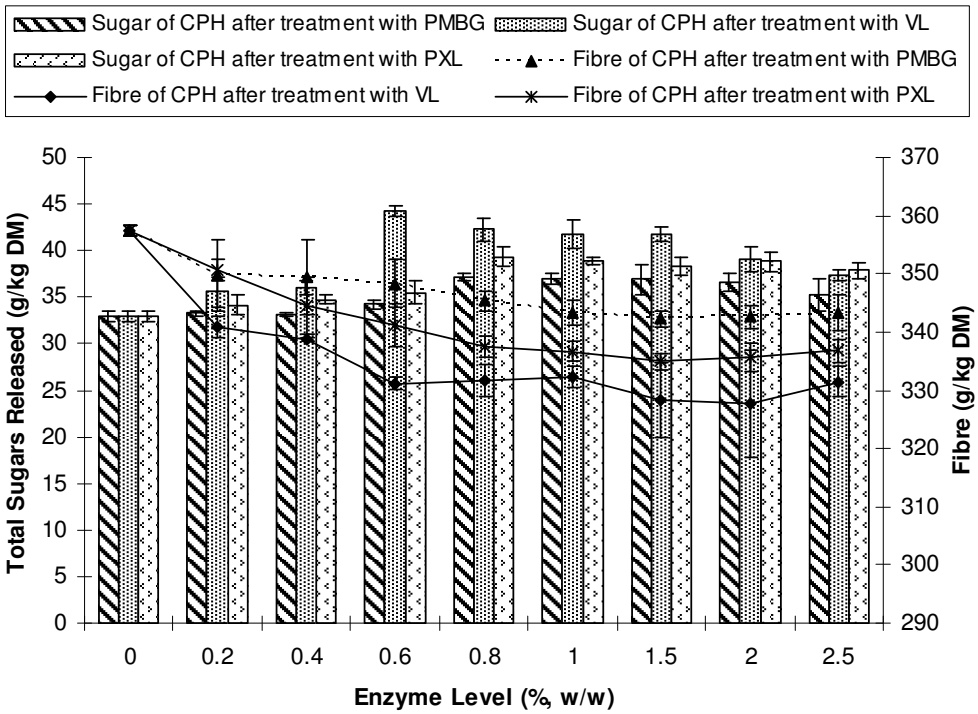

Figure 1. Effect of varying concentrations of Pentopan®Mono BG, Viscozyme®L and Pectinex®5XL on Saccharification of Cocoa Pod Husk.
CPH = Cocoa Pod Husk.
PMBG = Pentopan®Mono BG.
PXL = Pectinex®5XL.
VL = Viscozyme®L.

Table 2. Effect of combined enzyme treatments on cocoa pod husk (CPH) fibre fractions and total sugars released.

Treatment	Component (g/kg DM)						
	Total sugars	Crude Fibre	NDF	ADF	Hemicellulose	Cellulose	Lignin
Untreated CPH (control)	^{a}33.0 ± 0.6	^{a}357.4 ± 0.9	^{a}597.9 ± 15.8	^{a}470.4 ± 6.2	^{a}127.5 ± 9.6	^{a}261.5 ± 3.0	^{a}211.6 ± 2.6
PMBG + VL	^{bc}47.1 ± 0.9 (42.73)	^{cd}330.1 ± 5.2 (-7.64)	^{b}550.8 ± 5.5 (-7.88)	^{b}442.9 ± 0.4 (-5.85)	^{bc}108.0 ± 5.2 (-15.29)	^{b}231.3 ± 2.9 (-11.55)	^{a}208.9 ± 3.2 (-1.28)
PMBG + PXL	^{b}45.2 ± 1.1 (36.97)	^{bc}335.8 ± 5.3 (-6.04)	^{ab}572.4 ± 8.6 (-4.27)	^{ab}458.9 ± 11.2 (-2.45)	^{ab}113.6 ± 2.6 (-10.90)	^{a}250.9 ± 7.6 (-4.05)	^{a}208.0 ± 3.7 (-1.70)
VL + PXL	^{cd}49.0 ± 1.2 (48.49)	^{de}311.2 ± 0.5 (-12.93)	^{c}529.6 ± 13.8 (-11.42)	^{c}422.0 ± 9.2 (-10.29)	^{bc}107.6 ± 4.6 (-15.61)	^{c}215.7 ± 7.7 (-17.51)	^{a}206.3 ± 1.5 (-2.51)
PMBG + VL + PXL	^{d}50.7 ± 0.4 (53.64)	^{e}307.1 ± 8.7 (-14.07)	^{c}513.9 ± 3.8 (-14.05)	^{c}418.2 ± 3.0 (-11.10)	^{c}95.6 ± 0.8 (-24.98)	^{c}214.8 ± 0.8 (-17.86)	^{a}203.5 ± 3.8 (-3.82)

Values are presented as Mean ± Standard deviation. Values in brackets represent % change from control. Values in the same column with same superscript are not significantly different (P > 0.05).
PXL = Pectinex®5XL.
PMBG = Pentopan®Mono BG.
VL = Viscozyme®L.

in plant cell wall and can covalently tie up polysaccharides such as hemicellulose and cellulose forming lignocellulose complex (Kirk and Farell, 1987; Kuhad et al., 1997). High lignin and high crystallinity of cellulose are reported as major barriers that can restrict enzyme action on NSPs in plant feedstuffs (Beldman et al., 1987).

General implications of study

The present *in vitro* study generally showed that addition of an effective blend of commercial extracellular exogenous fibrolytic enzymes improves cell wall digestion of CPH, and thus has the potential of enhancing the digestive capacity of poultry to efficiently utilise CPH based diets. On-farm growth performance study needs to be conducted to further support the present results. This is important because it can be argued that the versatility and efficacy of an exogenous multi-enzyme activity is related to its ability to adapt to the physiological conditions of the digestive tract of poultry birds.

In addition, the degree of improvement obtained by adding enzymes to the diet will depend on the age of the animal (young or adult). Positive *in vivo* performance results following NSP-degrading enzyme supplementation would have beneficial impacts on animal production as it would, among others, allow feed manufacturers the greater flexibility of using less expensive CPH as ingredient in the formulation of diets for poultry. These concerns may also apply to other monogastric livestock such as pigs. The *in vivo* effectiveness of one of the blends, VL + PXL, PMBG + VL + PXL and PMBG + VL, would soon be verified in a broiler feeding study.

ACKNOWLEDGEMENTS

Cocoa Research Institute of Ghana, New Tafo-Akim is acknowledged for providing dried cocoa pod husk pellets for the research. The authors are also grateful to Dr. Isaac Ashie of Novozymes Raleigh, N.C., USA, for providing the enzyme preparations.

Abbreviations: CPH, Cocoa Pod Husk; **NSP**, Non-Starch Polysaccharides; **PMBG**, Pentopan®Mono BG; **PXL**, Pectinex®5XL; **VL**, Viscozyme®L.

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