Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Biology

Elixir Appl. Biology 49 (2012) 10119-10123

ISSN: 2229-712X

Changes in the Nutritional Quality of Corn Offal Fermented with *Penicillium notatum* and *Penicillium citrinum*

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ARTICLE INFO

Article history:

Received: 21 June 2012;

Received in revised form:

16 August 2012;

Accepted: 24 August 2012;

Keywords

corn offal,

Penicillium notatum,

Penicillium citrinum,

In vitro digestibility,

Chemical composition.

ABSTRACT

This study was carried out to evaluate the changes in nutritional quality of corn offal (CO) fermented with *Penicillium notatum* and *Penicillium citrinum* using in vitro gas production technique. After the fungal biodegradation of CO in a solid state fermentation, the chemical composition and in vitro digestibility of the undegraded and the degraded CO were determined. It was observed that there were significant (P<0.05) differences in the crude fibre values. The crude fibre (CF) significantly (P>0.05) decreased. It decreased from 8.02g/100gDM for the control (UC) to 5.41g/100gDM for *Penicillium notatum* degraded corn offal (PNC) and 6.22g/100gDM for *Penicillium citrinum* degraded corn offal (PCC). The results also showed significant (P<0.05) differences in the values for detergent fibres. There was a significant (P<0.05) increase in the crude protein (CP) contents from 10.69g/100gDM for the control (UCO) to 16.69g/100gDM for PCC and 14.41g/100gDM for PNC. The value of organic matter digestibility (OMD) was from 28.46 to 62.28%, metabolizable energy (ME) improved from 4.59 to 10.18MJ/kg DM and short chain fatty acid (SCFA) values ranged from 0.03 to 0.85µM. There were significant (P<0.05) differences in the values obtained for potentially degradable fractions (b) ML. Rate of degradation of b, that is, C, was faster in the degraded CO compared with the undegraded CO. The obtained results revealed the possibility of using fungal treatment for the improvement of nutritional quality of corn offal for ruminant nutrition.

Introduction

The population of the world is increasing at a very high rate and most of the population growth is apparent in the developing countries. With the increasing demand for livestock products as a result of rapid growth in the world population, future hope of feeding the millions and protecting their food security will depend on the better utilization of non-conventional feed resources, which cannot be used as food for humans. Non conventional feed ingredients are by-products which are obtained during harvesting or processing of a commodity in which human food or fibre is derived (Makkar *et al*., 1995). In developing countries, ruminants are fed low quality roughages in various proportions depending on the type of animal and season. The main components of agro-industrial residues (cellulose, hemicellulose and lignin) are complex and its biodegradability is low, due to their resistance to biodegradation by ruminal microorganisms (Jarrige *et al.,* 1995).However, biotechnology could offer possibility of modifying the chemical structure of these substrates and thereby improve their digestibility. Some efforts have been made to apply biotechnological processes to the improvement of the nutritious quality of agro-industrial by-products like ruminant feed. Akinfemi *et al.,* (2009) treated maize cobs using *Pleurotus pulmonarius* and *Pleurotus sajor caju* in solid state fermentation (SSF).They found that after *P. pulmonarius* fermentation, protein and mineral content increased 52.02 and 15.67%, respectively, accompanied by an 42.09% increase in digestibility while fermentation with *P. sajor caju* achieved 52.05 and 11.50% improvement in crude protein and ash respectively after 21 days of fermentation. Bauer *et al*., (2003) reported the effect of enzymatic treatments on several substrates rich in carbohydrates, showing that the fermentabilities of the enzyme-treated and untreated substrates were different (Guadalupe Ortiz-Tovar et al., 2007). The use of solid-state fermentation (SSF) to upgrade the nutritive value of agricultural by-products and for enzyme production has been increased due to the higher quantity of residues produced in several countries (~3.5 billion tones per year), representing a potential solution to feeding animals in developing countries (Robinson and Nigam, 2003). The advantage of using SSF to achieve both goals is low-tech fermentation system required plus the possibility of having it carried out on farm.SSF can be said to be a fermentation process in which micro-organisms grow on solid materials without the presence of free liquid (Pandey, 2003). CO is the feathery seed coat removed from the back of corn seeds. That is, CO is the seed coat of corn. It is available in large quantities in many equatorial countries of Africa, Asia, and America. CO is used at moderate level in animal feed due to its high fibre content as well as low essential amino acids (Lysine).In SSF, the moisture necessary for microbial growth exists in an absorbed state or complexed within the solid matrix. The use of *in vitro* technique is rapidly expanding because of an increased need for reproducible methods to obtain data on

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bioavailability of nutrients in addition to chemical ones (Williams, 2000). Since digestibility trials are expensive and laborious to perform, there have been numerous attempts to determine the digestibility of feeds by reproducing in the laboratory the reactions which take place in the alimentary tract of the animal (H¨olke, 2005).The purpose of this study is to evaluate the effect of *Penicillium notatum* and *Penicillium citrinum* on the nutritional quality of Corn offal through chemical analysis and in vitro gas production technique.

Materials and Methods

Fungi

The fungi used in the study were *P. notatum* and *P. citrinum sp.* Slants of the microbes were obtained from the Department of Biological Science (Microbiology Unit), Bowen University, Nigeria. A portion of mycelia of each of the fungi was then subcultured on potato dextrose agar (PDA) in Petri dishes and incubated at 30^{o}C for 4 days (Iyayi, 2004).

Corn offal as the substrate

CO was obtained from feed miller in Ibadan, Nigeria. Enough quantity was bought at the commencement of the experiment to prevent fluctuation in quality with different batches because of the inconsistent processing techniques as observed by Onilude and Oso (1999). 50g of milled sample of CO was placed in 250ml Erlenmeyer flask. The mouth of the flask were clogged with cotton wool and then covered with aluminum foil. The flasks containing the substrates were autoclaved at 12^{o}C for 15 minutes. After autoclaving, it was moistened with 20 ml of basal medium containing :1.4g NH_4Cl, 0.3g urea , 2.0g KH_2PO_4, 1.4g $(NH4)_2SO_4$, 0.3g $MgSO_4.7H_2O$, 0.4g $CaCl_2.2H_2O$, 0.5mg Nicotinic acid, 0.5mg Riboflavin, 0.05mgThiamine and Biotin per litre of distilled water to obtain humidity of 38%. Flasks were inoculated with 1.0ml of aqueous spore suspensions of the respective fungus. Three set of flasks each containing CO was aseptically inoculated with each of the 2 fungi. Another set of 3 flasks containing the autoclaved CO was uninnoculated. This was done in order to ensure that each treatment was replicated. The flasks were covered back with sterilized cotton wool and placed in incubator set at 35^{o}C. Samples were withdrawn on the 7th day and the action of the microbes was terminated by drying at 60^{o}C. The samples were dried to constant weight, thoroughly mixed and stored in sterilized containers for chemical analysis and *in vitro* digestibility studies.

***In vitro* gas production**

The rumen fluid was collected from six rams before morning feed was administered. The fluid was collected through the suction method (by means of the suction tube or hose) from six rams under the same feeding regime. The animals were fed according to their body weights; they were fed with 60% guinea grass and 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soya bean meal, 10% dried brewer grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal). The collected rumen fluid was filtered through a four-layered cheese cloth into a flask flushed with carbon dioxide (CO_2) gas and stirred using an automatic stirrer. Incubation was carried out using the method of Menke and Steingass (1988).The buffer solution prepared was the McDougall's buffer containing:

$NaHCO_3$+Na_2HPO_4+KCl+NaCl+$MgSO_4.7H_2O$+$CaCl_2.2H_2O$ in a ratio (1:4 v/v). 30ml of the innoculum was introduced into each of the pre-warmed syringes through the silicon tubes. Air bubbles trapped in the syringes were removed by shaking the syringe and then pushing the piston upward after which the steel clip on the tubes were screwed tightly. Blanks were also prepared with 30ml of innoculum, without the feed sample. The blanks were prepared as control. The incubation period lasted for 72 hours and the gas production was observed and recorded at an interval of 3 hours, including the blanks. Gas production was terminated at the end of the first 24hours, 48 hours and finally 72 hours. After each termination, 10 molar solution of NaOH was introduced through the silicon tube. After opening the steel clip, the mixture was thoroughly shaken; NaOH absorbed the CO_2 gas present in the syringe, leaving only the methane gas. The volume of methane gas was recorded and the volume of the CO_2 was determined by subtracting the volume of methane from total gas produced. Data of the *in vitro* gas production were analyzed using the equation $Y=a+b\ (1-e^{-ct})$ described by Ørskov and McDonald (1979) where Y=volume of gas produced at time t, a=intercept (gas produced from the soluble fraction), b=gas produced from the potentially degradable fraction, (a+b) = final gas produced, c=rate of degradation of b, t=incubation time. Metabolizable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) were estimated as established (Menke and Steingass 1998) and short chain fatty acids (SCFA) was calculated as reported Getachew *et al* (1999).

ME = 2.20 + 0.136GV + 0.057CP + 0.0029CF

OMD = 14.88 + 0.88GV + 0.45CP + 0.651XA

SCFA=0.0239GV+0.0601

Where GV, CP, CF and XA are: net gas production (ml/200mg, DM) crude protein, crude fibre and ash of the incubated sample respectively.

Chemical composition

The proximate analysis was carried out according to method of AOAC (1995).To determine the dry matter, 50g of the samples were weighed after drying at the termination of fermentation. The weighed samples were wrapped in weighed foil papers and dried at 105^{o}C to a constant weight for 24 hours. Nitrogen (N) content of the milled dried samples was determined by the standard Kjeldhal method (AOAC 1995) and the crude protein (CP) was calculated (N x 6.25).Ash content was determined using muffle furnace. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) was determined using the method described by Van Soest *et al*., (1991).Hemicellulose was estimated as the difference between NDF and ADF, and cellulose estimated as the difference between ADF and AD.The nitrogen free extract (NFE) was then derived by calculation :100-(CP+CF+ash +ether extract).

Statistical analysis

The obtained were subjected to analysis of variance (ANOVA) according to the procedure of Steel and Torrie (1980) and means were separated by Duncan's multiple range test where there were significant differences using Statistical Analysis System (SAS) 1999 package.

Result and discussion

Changes in chemical composition

Table 1: Chemical composition (g/100gDM) of *Penicillium notatum* and *Penicillium citrinum* degraded corn offal and undegraded corn offal

PARAMETERS	PNC	PCC	UCO	SEM
Dry matter	93.18	93.34	93.10	0.11
Ash	5.00^{a}	5.50^{b}	4.00^{b}	0.34
Crude fiber	5.41^{c}	6.22^{b}	8.02^{a}	0.01
Crude protein	14.41^{b}	16.22^{a}	10.69^{c}	0.17

Acid detergent fiber	33.92[b]	32.12[c]	35.00[a]	0.12
Acid detergent lignin	3.64[c]	4.13[b]	5.00[a]	0.06
Neutral detergent fiber	40.00[b]	40.50[b]	52.50[a]	0.08
Cellulose	31.53[b]	31.46[b]	36.11[a]	0.02
Hemicellulose	18.78[b]	18.36[b]	20.74[a]	0.02

a ,b,c, means on the same column with different superscripts are significantly varied (P < 0.05) PNC = Penicillium notatum degraded corn offal, PCC = Penicillium citrinum degraded corn offal, UCO=Control; it is the undegraded CO. SEM= standard error of the mean

The result of chemical composition of the treated and untreated maize cob is given in Table 1. There were variations in the chemical compositions of the degraded and the undegraded corn offal. The CP improved from 10.69 to 16.22g/100DM, the ash (minerals) also improved from 4.00 to 5.50g/100DM. There were significant (P<0.05) differences observed in the CF, cellulose, hemicellulose, neutral detergent fibre (NDF), acid detergent lignin (ADL) and acid detergent fibre (ADF). There were no significant (P>0.05) differences in the dry matter. The highest value of CF (8.02g/100DM) was obtained in the undegraded CO. The significant increase in the CP after fungal biodegradation of CO can be traced to the fungal growth, which increase the hydrolysis of starch to glucose and its subsequent use by same organisms as a carbon source to synthesize fungal biomass rich in protein(Akinfemi et al, 2009).Besides, fungi have the ability to produce a variety of enzymes. These enzymes help to degrade the non-starch polysaccharides (NSPs) in the substrates. The produced enzymes break the cell walls of the fibrous materials and thereby reduce the crude and the detergent fibres. Iyayi and Aderolu (2004) reported reduction in crude fibre content of brewer dried grain (BDG), maize bran (MB) and wheat offal (WB) when A. *niger, A. flavus* and *Penicillium sp* were cultured on them. According to these workers, crude fibre in the above mentioned AIBs were significantly (P<0.05) reduced by all the fungi after 14 days. *A niger* consistently caused the highest reduction in crude fibre in all the AlBs followed by *A. flavus* and *Penicillium sp.* The highest percentage reduction by *A. niger* was achieved in WB (36.51%) followed by BDG (35.87%) and MB (35.80%). The ability of fungi to degrade crude fibre has also been reported by Ofuya and Nwajiuba (1990), Iyayi and Losel, (200l). Earlier works of the latter showed successful degradation of cassava peel by *Rhizopus spp.* Akinfemi et al (2009) opined that lignifications of structural polysaccharides not only inhibit ruminal microbial digestion of polysaccharides by forming 3-D matrix, but also that the presence of highly lignified tissues forms a physical barrier preventing accessibility of the otherwise highly digestible tissue to the action of hydrolytic enzymes of the rumen micro-organisms. Fungal biodegradation of corn offal therefore has the potential of breaking the structural carbohydrates and thereby increase its digestibility by the ruminant animals.

Organic matter digestibility (OMD), short chain fatty acid (SCFA), metabolizable energy (ME) and methane (CH_4) production

Table 2: **Organic matter digestibility (%), short chain fatty acid (µmol) and metabolizable energy (MJ/Kg DM) of degraded and undegraded corn offal**

PARAMETERS	PNC	PCC	UCO	SEM
Organic matter digestibility (%)	46.56[b]	50.53[a]	39.79[c]	0.15
Short chain fatty acid (µmol)	0.32[b]	0.52[a]	0.23[c]	0.03
Metabolizable energy (MJ/Kg DM)	7.97[a]	8.08[a]	6.76[b]	0.19

a, b, c, means on the same column with different superscripts are significantly varied (P < 0.05) PNC = Penicillium notatum degraded corn offal, PCC = Penicillium citrinum degraded corn offal, UCO=Control; it is the undegraded CO. SEM= standard error of the mean

Table 2 shows the values for organic matter digestibility (OMD), short chain fatty acid (SCFA) and metabolizable energy (ME) and methane gas production. There were significant (P<0.05) differences in the values for PNC, PCC and UCO. The highest value for OMD (50.53%) was obtained in corn offal degraded with *Penicillium citrinum* followed by the value obtained for Penicillium notatum degraded corn offal (46.56%). In their work, Akinfemi et al (2009) degraded maize cob with *Pleurotus sajor caju* and *Pleurotus pulmonarius*. It was observed that maize cob degraded had higher values with the highest value (42.09%) found in *Pleurotus pulmonarius* degraded maize cob. The higher OMD obtained from fungal degraded CO suggests that the microorganisms in the animals can possibly increase the nutrient availability Getachew *et al.*, (2004). The Metabolizable energy values were 7.97, 8.08 and 6.76MJ/Kg DM for PNC, PCC and UCO respectively. According to Gilbert and Hazelwood (1993), fungal biodegradation can increase the ME of agro industrial by products as it leads to an increase in the digestibility of cell wall components and this also enhances the starch digestibility. Breakdown of β-glucan via fungi can result in more efficient starch utilization and hence increase metabolizable energy. The *in vitro* gas production method had been employed by many workers to determine the energy value of feeds (Getachew *et al* 1999; Getachew *et al* 2004). According to Krishnamoorthy *et al.*, (1995), *in vitro* gas production technique may be considered for estimating ME in tropical feedstuffs; it is not laborious and less costly. SCFA was highest in *Penicillium citrinum* degraded corn offal (0.52µmol) and least in the undegraded corn offal (0.23µmol). According to Akinfemi et al (2009), SCFA indicates the energy available to the animals; it contributes up to 80% of animal daily energy requirements. The higher SCFA in the treated substrates might be due to increase in the crude protein and reduction in the crude fibre. SCFA indicates the energy available to the animal. Methane was significantly reduced in the fungi degraded corn offal, (Figure 1). Methane gas is an important gas among gases produced by ruminants at fermentation and it has been reported to be an energy loss to the animals and when emitted, it contributes to the destruction of ozone layer. Besides, when the dry matter degradation occurs in rumen by the action of microorganisms, there is production of gas which mainly constitutes hydrogen, carbon dioxide and methane. When it accumulates in the rumen, it results in bloat (Babayemi and Bamikole 2006, Babayemi et al 2006).

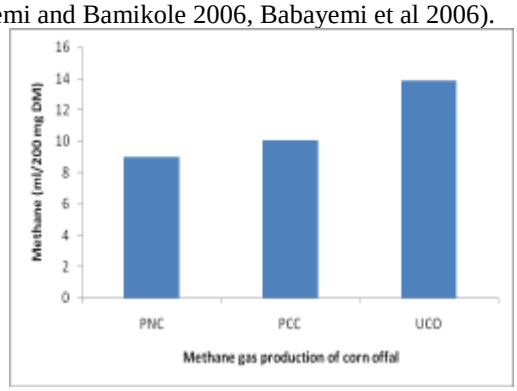

Figure 1: Methane (ml/200mgDM) production of corn offal

Gas production characteristics

Table 3: In vitro gas production characteristics of degraded and undegraded corn offal

PARAMETERS	PNC	PCC	UCO	SEM
a	1.44^{b}	2.03^{a}	2.22^{a}	0.24
b	22.39^{c}	32.63^{b}	35.11^{a}	1.37
a+b	23.83^{c}	34.66^{b}	37.33^{a}	1.00
$c(h^{-1})$	0.16^{a}	0.17^{a}	0.14^{b}	0.02
t	9.67^{a}	9.25^{a}	7.78^{b}	0.42
y	6.27^{b}	9.66^{a}	8.33^{a}	1.01

a, b, c, means on the same row with different superscripts are significantly varied (P< 0.05), PNC = Penicillium notatum degraded corn offal, PCC = Penicillium citrinum degraded corn offal, UCO=Control; it is the undegraded CO. SEM= standard error of the mean, (a+b) = Potential extent of degradation, b= fermentation of the insoluble but degradable fraction, y= volume of gas produced, c= Rates of gas production

There were significant (P<0.05) differences in the values of: a (gas produced from the soluble fraction), b (gas production from the insoluble fraction), a+b, c (gas production rate constant for the insoluble fraction, t (incubation time) and y (volume of gas produced at time t). The undegraded CO had highest value of b (35.11ml/500mg/DM). Lower values were observed in the degraded corn offal. This is in line with the observation of Guadalupe Ortiz-Tovar (2007) that possibility of having larger values are usually registered in substrates without treatment. A low value of b shows a high affinity of the rumen microorganisms for the substrate used. The parameter c measures the bioavailability of nutrients of a substrate in the rumen (Blummel and Becker, 1997). Therefore, a large value of c shows a high availability of substrates. This can be viewed as another form of affinity between rumen microorganisms and the substrates (Guadalupe Ortiz-Tovar, 2007). The results from this study are in line with those obtained by Bauer et al. (2003). These workers evaluated the effect of enzymatic treatments on several rich carbohydrates substrates using in vitro gas production. They found that enzymatic treatments applied to sugar cane pulp and wheat straw diminished a and increased b (P<0.05).

Gas volume

Table 4 shows the results of the in vitro gas production over a period of 24 hours.

Table 4: In vitro gas production from degraded and undegraded corn offal for 24 hours

Incubation period (Hours)	3	6	9	12	15	18	21	24
PNC	11.01^{a}	13.01^{b}	15.69^{ab}	16.22^{b}	17.88^{b}	19.98^{a}	20.43^{b}	22.41^{b}
PCC	11.13^{a}	14.82^{a}	16.84^{a}	18.64^{a}	19.74^{a}	21.11^{a}	22.96^{a}	24.12^{a}
UCO	9.45^{b}	10.32^{c}	13.02^{b}	14.12^{c}	15.21^{c}	16.53^{b}	18.21^{c}	20.20^{c}
SEM	0.75	0.66	0.51	0.44	0.24	0.36	0.41	0.37

a, b, c, means on the same column with different superscripts are significantly varied (P< 0.05), PNC = Penicillium notatum degraded corn offal, PCC = Penicillium citrinum degraded corn offal, UCO=Control; it is the undegraded CO. SEM= standard error of the mean

There were significant (P<0.05) differences observed in the final gas produced (24^{th} hour). The highest was in PCC (24.12%) followed by PNC (22.41%) and the least from UCO (20.20%). According to Rodrigues et al (2002), the higher the gas produced, the higher the short chain fatty acids. Gas production helps to measure digestion rate of soluble and insoluble fractions of feed stuff (Menke and Steingass, 1988).The gas produced is directly proportional to the rate at which substrate are degraded (France et al, 2000). Villas-Boas et al (2004) reported direct relationship between OMD and gas production. The quality of gas produced during fermentation reflects the amount of substrate digested and the microbial metabolic pathway.

Conclusion

1. The results showed that fungal biodegradation of corn offal with *Penicillium notatum* and *Penicillium citrinum* improved the crude protein content and reduced the detergent fibres and the crude fibre of the degraded corn offal.
2. There was an improvement in the in-vitro digestibility of the corn offal
3. Inclusion of biodegraded corn offal in the diet would be of benefit to ruminant livestock.

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