Vol. 28 (no. 2, June 2016) pp 96–105

Biokemistri

Nigerian Society for Experimental Biology (http://nisebpublications.org)

Research Article

Bioconversion of *Aspergillus niger KM* treated Rice and Wheat Bran for Experimental Rat Feed Formulation

Faoziyat A. Sulaiman[1], Muinat O. Kazeem[2,5], Musbau A. Akanji[1], Abdulfattah A. Sulaiman[3], Ogechukwu I. Igwe[1], Ridwan O. Asunmogejo[1], Williams I. Oluwafemi[1], Abiola A. Anifowose[2], Oluyomi S. Adeyemi[4], Adisa M. Jimoh[5], and Nor Aini Abdul Rahman[6].

[1]Department of Biochemistry, Faculty of Life Sciences, PMB 1515, University of Ilorin, Nigeria.

[2]Department of Microbiology, Faculty of Life Sciences, PMB 1515, University of Ilorin, Nigeria.

[3]Federal Medical Centre, Ebute Metta, Lagos, Nigeria.

[4]Department of Biological Sciences, College of Sciences & Engineering, Landmark University, Omu Aran, Nigeria.

[5]Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria.

[6]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra, Malaysia.

Correspondence: Faoziyat Sulaiman; faoziyat20022002@yahoo.com, +2348032127200

ABSTRACT: **This study investigated the nutritional value of pre-treated rice and wheat bran wastes as feed formulation in experimental rats. *Aspergillus niger KM* isolated from decomposing organic matter was tested for cellulase assay. The lignocellulosic agricultural residues (wheat bran and rice bran) were pretreated with ammonia and diluted sulfuric acid after which solid substrate fermentation with *Aspergillus niger KM* was carried out. Determination of reducing sugar was carried out and the fermented residues were included as components in feed formulation and were fed to different groups of rats for four weeks. The proximate analysis of the feed formulation showed that the NH_3 pretreated feeds gave higher protein content of 21.94%, relative to the control or other groups. Growth performances of animals fed with NH_3 pretreated wheat bran significantly increased from 158.25 to 201.66 g throughout the feeding periods. Evaluation of the various feeds' effect on tissue marker enzymes revealed inconsistent alterations relative to the control. Bioconverted wheat or rice bran has nutritive value to support animal growth and could be explored in animal feed preparation.**

KEYWORDS: **Animal feeds; Reducing sugar; Pre-treatment, Agricultural residues; Microbial fermentation.**

Received: 6 June 2016: Approved: 20 June 2016.

BKM.2016.016 © 2016 Nigerian Society for Experimental Biology; All rights reserved.
This article is available online in PDF format at http://nisebpublications.org and http://www.bioline.org.br/bk

INTRODUCTION

Lignocellulosic biomass contain the highest amount of fermentable carbohydrate from renewable sources on earth (Mtui and Nakamura, 2005; Ahmadi *et al*., 2010). Wheat bran and rice bran like most agricultural residues are rich in cellulose and hemicelluloses but unfortunately deprived of protein, which restricts their use as perfect animal feed. Improvement in their nutritional quality is therefore necessary for their full utilization. Soluble sugars released as a result of hydrolysis of cellulose create availability in feedstock and use in alcohol fermentation, production of single cell proteins and chemicals of industrial importance (Biag *et al*., 2004; Louime and Uckelmann, 2008).

Considering various fermentation processes, Solid State Fermentation (SSF) have proved to be an appropriate process for the production of various useful products from agro-industrial residues. The benefits have been found in the production of improved yield, low capital investment, low downstream processing, production of valuable products such as Single Cell Protein (SCP), enzymes and protein rich feeds from various agricultural wastes (Pandy *et al*., 2000, 2001; Pandy, 2003).

Structural unlock and removal of secondary bonds linking glucose chains are the purpose of lignocellulosic pre-treatments. Lignin removal can be achieved by alkali pre-treatment. Treatment with alkali would remove lignin, thus hydrolysis made easy. Hence, recovery of glucose from cellulose is accomplished. Extracellular cellulase enzymes from fungi break down cellulose into two or three glucose units that are easily reduced to glucose monomers (Chinedu *et al*., 2010).

An array of hydrolytic enzyme produced by *Aspergillus niger* are valuable for degradation of polysaccharides which are useful in food and feed industry (De Vries and Visser 2001; Jahromi *et al*., 2010). *Aspergillus niger* has been utilized in various industrial processes like amylase, cellulase, feruloyl esterase and phytase production.

This study attempt to examine the *Aspergillus niger KM* treated rice and wheat brans as nutritional components of experimental animal feeds. The formulated animal feeds were also evaluated for their effect on growth and other biochemical parameters.

MATERIALS AND METHODS

All reagents used were of analytical grade and prepared with distilled water except otherwise stated. Rice bran and wheat bran were gotten from Roffat Feed Company, GSS, Ilorin, Kwara State, Nigeria.

Soil samples collection and isolation of fungi

Three soil samples were collected; a plant root, woody soil and a scoop of decayed plant matter, which were collected from the agricultural farm land of University of Ilorin, Kwara State, Nigeria. (10N, 8E). Each soil samples were serially diluted (10^{-3}) using sterile distilled water. One g of each soil samples was added into a test tube containing 9 ml of distilled water and the serial dilution was done. 1 ml from 10^{-2} and 10^{-3} was inoculated into separate petri dishes, followed by addition of sterilized potato dextrose agar (PDA). The culture medium was whirled and the plates were incubated at 25^{o}C for 72 hrs. After 72 hrs, the plates were observed for fungal growth and sub-cultured.

Morphological and microscopic examination

After the 5th day, a more distinct and visible growth of fungi was observed. Numerous fungal species suspected to be *Aspergillus sp. and Trichoderma sp* were found and were further sub-cultured to obtain pure cultures. The pure cultures were sub-cultured on PDA slant for further analysis. The fungi species were observed by colour, arrangement of hyphae and spores was also examined with methylene blue under the microscope and identified using the compendium of fungi. Both organisms ware subjected to cellulase assay and *Aspergillus sp KM was* chosen based on the highest zone of clearance.

Screening for cellulase activity

A plate assay method described by Hankin and Anagnostakis (1975) was used. Briefly, 1% carboxymethylcellulose (CMC) was prepared in a basal salt medium. *Aspergillus sp KM* was inoculated by streaking and the plate was incubated at 25^{0}C for 48 hrs. The fungi was tested for its ability to produce cellullase (required to degrade the CMC) by adding 0.1% Congo red solution, counterstained with 1 M NaCl and incubated at 25^{o}C for 20 minutes. Hydrolysis of cellulose appeared as a clear zone around the colony.

Substrate Pre-treatment Procedures

Ammonia pre-treatment

Ammonia pre-treatment was performed following the procedure obtained by Ko *et al*. (2009) 1000 g of the substrates (Wheat or rice bran) was weighed and was divided into equal proportions of 66.7 g into 15 bottles. using a (1:6) solid-liquid ratio and 21%w/w NH_3. The mixture was then placed in the oven at 69^{o}C for 10 hours. After which the mixture was washed with sterile distilled water to remove excess ammonia and then dried at 50 ^{o}C.

Table 1: Feed composition and formulation

COMPOSITION	CONTROL	GROUP 1 (NH_3)	GROUP 2 (NH_3)	GROUP 3 (H_2SO_4)	GROUP 4 (H_2SO_4)
Sucrose	100 g	100 g	100 g	100 g	100 g
Cellulose	40 g	40 g	40 g	40 g	40 g
Soybean	250 g	250 g	250 g	250 g	250 g
Vitamin/mineral mix	50 g	50 g	50 g	50 g	50 g
D-methionine	4 g	4 g	4 g	4 g	4 g
Rice bran	-	506 g	-	506 g	-
Wheat bran	-	-	506 g	-	506 g
Cornstarch	506 g	-	-	-	-
Soybean oil	50 ml	50 ml	50 ml	50 ml	50 ml

Sulphuric acid pre-treatment

The sulphuric acid pre-treament followed the protocol described by Linde *et al.* (2009). Briefly, using a loading ratio (10:90) Solid-liquid ratio; 18 ml of concentrated H_2SO_4 was measured into 8.9 litres of distilled water. 1000 g of the substrate (wheat or rice bran) was weighed and divided into equal proportions i.e. 66.7g into 15 bottles. The resulting solution was poured in equal volume (594 ml) into the 15 bottles containing the substrate and stirred. The mixture was then placed in the oven at 90 ^{o}C for 10 minutes. After 10 minutes, the mixture was allowed to cool. Residual acid was removed by washing with distilled water, and then dried at 50^{0}C.

Maintenance of pure culture and inoculum preparation

Aspergillus sp KM was maintained on Potato-Dextrose Agar (PDA) slant and stored at 4^{0}C for further use. The fungi was sub-cultured into PDA slant and incubated at 25^{0}C for 7 days to allow for spore production. The surface of the slant was washed with 10 ml sterile distilled water and used as inoculum.

Solid state fermentation

A total of 101 g of the differently pre-treated substrates (NH_3 pre-treated WB, NH_3 pre-treated RB, H_2SO_4 pre-treated WB and H_2SO_4 pre-treated RB) was weighted into bottles. Mendel's medium (Mandel *et al*., 1974) was added to give a moisture content of 70%. Major elements of the media include Urea 0.3g/l ; $(NH_4)_2SO_4$ 1.4g/l, $KHPO_4$ 2.0g/l; $CaCl_2 7H_2O$ 0.4g/l; $MgSO_4.7H_2O$ 0.3g/l; Peptone 0.75.

Trace elements include $FeSO_4.7H_2O$ 5.0mg/l; $MnSO_4.7H_2O$ 1.6mg/l; $ZnSO_4.7H_2O$ 1.4mg/l; $CoCl_2 .6H_2O$ 2.0mg/l. Fermentation was carried out at 30^{o}C for 96 hrs and initial pH adjustment at 4.8 and 1x10 ^{6} cfu/ml inoculum was used. 30 ml 0.2M acetate buffer pH 4.8 was added into each flask and shaken at 150 rpm for 30 minutes on an orbital shaker. Resulting homogenate was centrifuged at 4000 rpm at 4^{0}C for removal of the fungi cells and debris. The cell free extract was filtered for enzyme analysis. The fermented rice bran and wheat bran was autoclaved at 121^{0}C for 15 minutes before incorporation into the animal feed formulation.

Analytical procedure

To analyse exoglucanase (FPase) activity, filter paper was used as the substrate. Briefly, 0.5ml of the crude enzymes was added to Whatman no. 1 (1× 6cm; 50mg) dipped in 1 millilitre of 0.05M sodium citrate buffers at pH 5 following incubation at 50 ^{o}C for 60min. The reaction was stopped with 3mls DNS reagent and mixing (Adney and Baker, 1996). One unit of FPase activity is defined as the amount of enzyme releasing 1 micromole of reducing sugar per minute under assay condition. Endoglucanase (CMase) activity was measured in a reaction mixture of 1ml of 1% carboxymethyl cellulose (CMC) in 0.1 citrate acetate buffer (pH 5), mixed with 1 ml of the crude enzyme (Miller *et al*., 1960). Incubation was allowed for 20min at 50^{o}C and stopped with 3mls of DNS reagent and mixing. One unit of (endoglucanase) activity was defined as the amount of enzyme releasing 1micromole of sugar per minute under assay condition.

Experimental animals

Wistar rats weighing between 170 – 200 g were obtained from the experimental animal holding unit of the Department of Biochemistry, University of Ilorin, Nigeria. The rats were kept in well-ventilated and hygienic environment. Before the commencement of the experiment, rats were subjected to one-week acclimatization, during which they had free intake of clean water and rat show *ad libitum.* The cages and feeding troughs were cleaned daily.

Grouping and feeding of experimental animals

Experimental rats were randomly selected and divided into five groups, A, B, C, D, and E (Table 1). They were fed with cornstarch based formulated feed (Group A); NH_3 pre-treated rice bran formulated feed (Group B); NH_3 pre-treated wheat bran formulated feed (Group C); H_2SO_4 pre-treated rice bran formulated feed (Group D); H_2SO_4 pre-treated wheat bran formulated feed (Group E), respectively for four weeks. Handling of experimental animals was consistent with ethical guidelines, as approved by the University of Ilorin Ethics Committee for scientific and medical research.

Proximate analysis

The formulated feed were analyzed for their moisture content, protein content, ash content, fat content, fibre content, dry matter and the carbohydrate content (nitrogen free extract, NFE) using the procedures described by the Association of Official Analytical Chemists (AOAC, 1990).

Protein determination

Protein content was determined using Kjeldahl method. Briefly, 2 g of sample was weighed into a digestion tube and 15 ml of concentrated H_2SO_4 was added. Kjedahl tablets were added and digestion process was carried out in a digestion or fume cupboard at 41^0C for 45 mins until the solution became clear. About 75 ml of distilled water was added to prevent solidifying after digestion. The tube was placed in the distilling unit and 50 ml of 40% sodium hydroxide was dispensed into the diluted solution and the digest distilled into 25 ml of 40% boric acid for 5 mins. The distillate was then titrated against 0.47 M HCl until the first grey colour is seen. A blank was run at first and the titre value was recorded (AOAC, 1990).

Crude fat determination

Extraction flask 250 ml was washed, dried and weighed. The soxhlet extractor with reflux condenser was fitted up and allowed through the condenser. 2 g of the sample was weighed into a filter paper, folded and transferred into a free fat extraction thimble and plugged lightly with a cotton wool. The thimble was placed in the extraction barrel with addition of petroleum added, until it siphons once in the flask directly below it, ensuring that all points were tight, the flask and reflux sample were heated for about 5-8 hrs.

On completion of extraction, the thimble was removed from the extraction barrel and dried. The barrel was then replaced and distilled off the solvent until the extraction flask was almost dried. The flask containing fat was detached and dried in the oven at a low temperature to evaporate the solvent completely. The flask plus fat was weighed (AOAC, 1990).

Moisture Content Determination

Two grammes of sample was weighed into a pre-weighed clean dry dish. The uncovered dry dish was placed in well ventilated oven maintained at 105 ± 2 °C. After 2 hrs, the lid was replaced to cover the dish and placed in a dessicator to cool; it was then weighed as quickly as possible. The procedure was repeated until a constant weight was obtained (AOAC, 1990).

Crude fibre determination

Two grammes of the sample was weighed into the fibre flask and 100 ml of 0.225 M H_2SO_4 was then added. The mixture was allowed to heat under reflux for 1hr with heating mantle and the hot mixture filtered through a fibre filter cloth. The filtrate obtained was discarded and the residue was returned to fibre flask. 100 ml of 0.313 M of NaOH was added and heated under reflux for another 1 hr. The mixture was further filtered through a sieve cloth and 100 ml of acetone was added to dissolve the organic constituents present. 50 ml hot water was used in washing and further sieved and transferred into the crucible. The crucible and the residue was oven dried at 105 °C, then cooled in the dessicator and weighed W_1. It was transferred to the muffle furnace and ashed at 550 °C for 4 hrs. This was followed by coolling in the dessicator and weighed to obtain the W_2. The percentage fibre was expressed as below:

$$\textbf{\% Crude fibre} = \frac{(W_1 — W_2) \times 100}{\text{Weight of sample}}$$

Carbohydrate content

Carbohydrate content of the sample was determined as:

100 − (%Protein + %Ash + %Fat + %Crude fibre + %Moisture content)

Preparation of serum and tissues homogenates

At the end of treatments, rats were sacrificed under slight diethylether anaesthetisation. A clean centrifuge tube was used in blood collection, then spinned at 4000 g for 10 minutes to obtain the serum (Heraeus Labofuge 300, Thermo Scientific, Hampshire, UK). Liver, kidney and heart tissues

were harvested, weighed and homogenized with teflon homogeniser (Sigma-Aldrich Chemie GmbH, Munich, Germany) in a cold 0.25 M sucrose solution (1:5 w/v). The homogenates were further centrifuged at 5000 g for 10 minutes for removal of unbroken particulates. Serum and tissue homogenates were further used for biochemical analyses.

Biochemical assays

Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were assayed using Randox assay kits (Reitman and Frenkel 1957; Schmidt and Schmidt, 1963; Rec. GSCC 1972). Absorbance readings were carried out using spectrophotometer (Spectrumlab 752s).

Data analysis

Mean of groups was calculated for each data and level of significant differences between means were evaluated using one way analysis of variance (ANOVA). Post-test analyses were done using the Duncan Multiple Range at 99.5% significant level (α= 0.05).

RESULTS

Corn starch (506 g) in the same ratio (Table 1) was replaced by H_2SO_4 and NH_3 pretreated wheat bran to form a treatment diet. Other nutrients incorporated into the formulated feed were sucrose, cellulose, soybean, vitamin/mineral mix, D-methionine and soybean oil, to ensure a balanced diet for the experimental rats.

Cellulase activity of fermented pre-treated residues

The enzyme produced was measured as a function of endoglucanase and exoglucanase activity. The enzyme activity among the treatment groups varies at the 96th hour of fermentation (Tables 2). Overall, both H_2SO_4 and NH_3 pre-treated wheat bran gave higher enzyme activity than the rice bran. NH_3 pre-treated wheat bran gave the highest exoglucanase activity (0.34U/ml). There is a reduction in endoglucanase and exoglucanase activity in pre-tereated rice bran. The lowest endoglucanase activity (0.01U/ml) was observed in NH_3 pre-treated rice bran.

Nutritional improvement of the formulated feeds

The analysis of proximate composition of the pre-treated fermented formulated feeds (Table 3) generally showed an increase in the moisture, ash, protein, fat, fibre and dry matter contents over the control except in NH_3 pretrated wheat bran (5.92% -moisture), H_2SO_4 pretreated rice bran (7.35% fat, 89.55% dry matter) and NH_3 pre-treated rice bran (8.73% fat, 90.79% dry matter), which gave lower moisture, fat and dry matter contents, respectively. However, the highest nitrogen free extract (63.20%) was observed in the control feed. Statistical analysis showed that the ash content was statistically higher with respect to the control group in all the formulated feeds.

Analysis of protein, fat and dry matter contents revealed that both H_2SO_4 and NH_3 pretreated wheat bran gave higher values compared to the other feeds. The highest protein, fat and dry matter contents of 21.94%, 15.57% and 94.0 %, respectively was observed in NH_3 pretreated wheat bran.

Table 2: Cellulase activities and glucose released from pretreated substrates after 96 hours of degradation by *Aspergillus sp KM*

Pretreated Substrates	Endoglucanase (U/ml)	Exoglucanase (U/ml)
H_2SO_4 pretreated Rice Bran	0.11	0.10
H_2SO_4 Pretreated Wheat Bran	0.17	0.12
NH_3 pretrated Rice Bran	0.01	0.04
NH_3 pretreated Wheat Bran	0.13	0.35

Table 3: Proximate composition of formulated feeds

Sample	Moisture content	Ash content	Protein content	Fat content	Fibre content	Dry matter	N_2-free extract
Control	7.23^{b}	4.16^{a}	14.50^{a}	8.98^{b}	1.93^{a}	92.77^{c}	63.20^{d}
NH_3 Wheat	5.92^{a}	8.02^{c}	21.94^{d}	15.57^{d}	10.95^{b}	94.08^{d}	37.60^{b}
NH_3 Rice	9.21^{c}	13.62^{e}	15.17^{b}	8.73^{b}	19.38^{d}	90.79^{b}	33.89^{a}
H_2SO_4Wheat	7.16^{b}	6.61^{b}	18.36^{c}	12.97^{c}	11.04^{c}	92.84^{c}	43.86^{c}
H_2SO_4 Rice	10.45^{d}	11.38^{d}	14.80^{a}	7.35^{a}	19.85^{d}	89.55^{a}	36.17^{b}

Values are percentage mean ± SEM (n = 3). Values with different superscripts in the same column are significantly different at $p<0.05$.

Table 4: Weight of experimental rats

Weeks	Control (g)	NH_3 Wheat (g)	NH_3 Rice (g)	H_2SO_4 Wheat (g)	H_2SO_4 Rice (g)
0	156.23 ± 21.13^{a}	158.25 ± 15.32^{a}	159.54 ± 15.50^{a}	156.33 ± 17.43^{a}	140.43 ± 6.42^{a}
1	161.13 ± 31.84^{c}	164.33 ± 18.67^{b}	152.00 ± 22.50^{b}	162.66 ± 25.41^{e}	146.63 ± 8.21^{a}
2	155.33 ± 33.33^{a}	177.00 ± 22.72^{c}	125.66 ± 20.49^{c}	146.00 ± 21.96^{c}	143.75 ± 8.33^{c}
3	151.66 ± 34.17^{b}	193.33 ± 29.48^{d}	118.66 ± 15.76^{d}	142.00 ± 22.27^{b}	138.50 ± 10.15^{a}
4	149.66 ± 33.22^{b}	201.66 ± 31.13^{e}	112.66 ± 20.21^{e}	137.00 ± 25.35^{a}	139.90 ± 11.43^{a}

Values are mean ± SEM (n = 3). Values with different superscripts in the same row are significantly different at $p<0.05$.

Table 5: Alkaline phosphatase (ALP) activity in rat serum and tissues

	Serum (U/l)	Kidney (U/l)	Liver (U/l)	Heart (U/l)
Control	3464.49±4.44^{e}	2855.10±7.23^{d}	2259.42±5.97^{a}	2156.78±7.79^{b}
NH_3 Wheat	1404.84±1.82^{a}	1719.24±4.69^{b}	2142.98±4.36^{a}	2332.50±1.82^{b}
NH_3 Rice	3239.62±9.90^{d}	1429.20±3.36^{a}	3392.03±7.03^{b}	2907.80±2.17^{d}
H_2SO_4 wheat	2345.69±5.50^{b}	2024.32±2.15^{c}	2259.21±6.78^{a}	1291.98±2.27^{a}
H_2SO_4 Rice	2938.49±1.14^{c}	1652.32±6.40^{b}	3309.24±8.33^{b}	2713.08±4.61^{e}

Values are mean ± SEM (n = 3). Values with different superscripts in the same column are significantly different at p<0.05.

No significant difference was observed in H_2SO_4 wheat moisture (7.16%) and dry matter content (92.84%) when compared with the control (7.23% and 92.77%). Rice bran pretreated with NH_3 produced the highest amount of ash content (13.62%), which was also significantly different from other formulated feeds.

Effect of feed on weight of rats

The weights of rats (Table 4) fed with NH_3 pretreated wheat bran increased significantly with increase in the number of weeks, with the highest weight (201.66g) recorded on the fourth week. In contrast, weights of rats fed with the other feeds increased slightly in the first week, followed by a decrease in the remaining weeks, except in NH_3 pretreated rice bran, which decreased as the feeding weeks progressed (159.54g – 112.66g). Rats fed with NH_3 rice bran had the least weight (112.66) by the end of the fourth feeding week.

Effect of feed on organ-body ratio

The organ-body weight ratios are as shown in Fig. 1 indicated that there was no significant difference among the various feed treatment groups. However, the liver of rats in groups 1 and 4 had the highest organ-body weight ratio when compared to the liver of group 2 rats.

Effect of feed on maker enzymes

The levels of marker enzymes including ALP, ALT and AST in rat serum and tissues were inconsistently altered relative to the control (Tables 5, 6 and 7). The least activities of ALP were observed in the serum (NH_3 Wheat), kidney (NH_3 Rice), liver (NH_3 Wheat) and the heart (H_2SO_4 wheat) when compared with the control group, while the highest activities were seen in the serum (NH_3 Rice), kidney (H_2SO_4 wheat), liver (NH_3 Rice) and the heart (NH_3 Rice). ALP activities in both the liver and heart of NH_3 rice group exceeds that of the control group (Table 5).

The least activities of ALT were observed in the serum (NH_3 Rice- 2.00U/l), kidney (NH_3 Wheat- 7.66U/l), liver (H_2SO_4 Wheat - 10.00U/l) and the heart (control- 9.33U/l) while the highest activities were seen in the serum (H_2SO_4 Rice and NH_3 Wheat- 12.00 U/l), kidney (H_2SO_4 wheat- 42.00 U/l), liver (H_2SO_4 Rice- 23.66 U/l U/l) and the heart (H_2SO_4 Rice- 18 U/l). ALT activities in both the liver and heart of H_2SO_4 Rice group significantly exceeds that of the control group (Table 6).

However, the highest activities of AST, when compared with the control group were observed in the serum (NH_3 Wheat- 58.66U/l), kidney (H_2SO_4 Wheat- 166.66), liver (H_2SO_4 Wheat-43.33) and the heart (H_2SO_4 rice- 61.33 U/l) while the least activities were observed in the serum (NH_3 Rice- 22.66 U/l), kidney (NH_3 wheat-15.00U/l), liver (control- 17.33U/l) and the heart (control- 25.00U/l). AST activities in both the liver (17.33U/l) and heart (25.00U/l) of control group are the least (Table 7) when compared with other groups 'activities.

Table 6: Alanine transaminase (ALT) activity in rat serum and tissues

	Serum (U/l)	Kidney (U/l)	Liver (U/l)	Heart (U/l)
Control	8.00 ± 2.30^{b}	9.33 ± 6.00^{a}	19.33 ± 3.84^{b}	9.33 ± 5.89^{a}
NH_3 Wheat	12.00 ± 0.00^{d}	7.66 ± 2.33^{b}	10.66 ± 9.17^{a}	13.33 ± 4.84^{c}
NH_3 Rice	2.00 ± 0.00^{a}	41.66 ± 4.09^{d}	21.00 ± 7.50^{a}	14.00 ± 10.00^{c}
H_2SO_4 Wheat	11.00 ± 2.00^{c}	42.00 ± 25.57^{d}	10.00 ± 4.58^{a}	11.00 ± 3.60^{b}
H_2SO_4 Rice	12.00 ± 5.56^{d}	39.66 ± 3.52^{c}	23.66 ± 10.08^{c}	18.00 ± 6.02^{d}

Values are mean ± SEM (n = 3). Values with different superscripts in the same column are significantly different at $p<0.05$.

Table 7: Aspartate transaminase (AST) activity in rat serum and tissues

	Serum (U/l)	Kidney (U/l)	Liver (U/l)	Heart (U/l)
Control	27.33 ± 12.54^{c}	24.33 ± 10.47^{b}	17.33 ± 14.83^{a}	25.00 ± 17.24^{a}
NH_3 Wheat	58.66 ± 4.91^{d}	15.00 ± 6.02^{a}	30.00 ± 11.00^{c}	44.00 ± 23.62^{b}
NH_3 Rice	22.66 ± 10.68^{a}	46.66 ± 22.98^{c}	18.66 ± 5.89^{a}	59.00 ± 15.09^{d}
H_2SO_4 Wheat	25.66 ± 8.19^{b}	166.66 ± 118.93^{d}	43.33 ± 23.62^{d}	51.66 ± 23.95^{c}
H_2SO_4 Rice	27.66 ± 12.34^{c}	28.66 ± 14.19^{b}	26.33 ± 14.19^{b}	61.33 ± 6.17^{e}

Values are mean ± SEM (n = 3). Values with different superscripts in the same column are significantly different at $p<0.05$

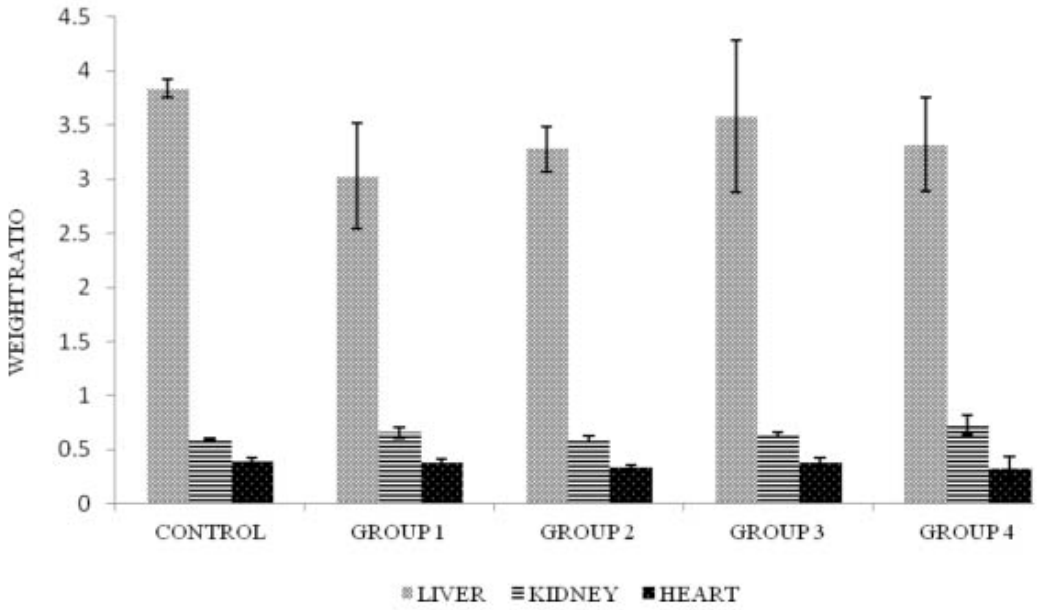


Figure 1: Organ body weight ratio of experimental rats. Values are presented as mean SEM (n=3). Group 1- control; Group 2 – ammonia pre-treated wheat bran; Group 3- sulphuric acid pre-treated wheat bran; Group 4- ammonia pre-treated rice bran; group 5- sulphuric acid pre-treated rice bran.

DISCUSSION

The activities of endoglucanase and exoglucanase as a function of sugar produced were evaluated. Both H_2SO_4 and NH_3 pre-treated were observed to favour endoglucanase and exoglucanase activities in wheat bran. This was due to the ability of the chemicals to break up the bonds in lignocelluloses, making cellulose accessible for microbial degradation. The reduction in the enzyme activity of pre-treated rice bran in this result is in agreement with that of (Rahnama *et al*., 2013) who recorded a higher cellulase activity in unpre-treated rice straw over 0.5% NaOH pre-treated one. This may be due to the reduction in the crystalline structure of the cellulose as a result of the pre-treatment (Sun *et al*., 2008).

The NH_3 and H_2SO_4 treated wheat feeds gave higher content of protein relative to the other groups. This could be attributed to the crude protein content in the wheat bran (Preston, 2006) coupled with that produced by *Aspergillus niger* during the fermentation. This result agrees with that of (Ahmadi *et al.,* 2010), who produced single cell protein from wheat straw treatment with Pleurotus Florida. Furthermore, the fat contents in the pretreated wheat feeds were higher compared to the control or the pretreated rice feeds. This could be due to 4.5% fat native to wheat bran (Preson, 2006). According to Linde *et al.* (2009), the pretreating agents (NH_3 and H_2SO_4) may help in delignification, thus allowing for the expression of protein and fat contents.

The significant increase ($p< 0.05$) in dry matter content of NH_3 pretreated wheat bran can be attributed to significant decrease in moisture content and this result confirm the earlier findings by Belewu and Sam (2010).

Conversely, the control feed had the highest content of carbohydrate. Since the control feed was made from corn starch, the higher carbohydrate content may be justified. The animals were fed for four weeks and their growth performances was assessed. The group fed with NH_3 wheat supplements had higher weights relative to the other groups. The reason for the variance in weight is unknown but the data suggest the capability of the NH_3 wheat feeds to support growth of animals over the other treatment groups. This could be as a result of phytase production which has increased the absorption of calcium. This result is in accordance with Bran (1999) who recorded an increased in calcium absorption by pigs fed with wheat phytase. Islam *et al.* (2009) also reported finishing lamb fed with diet containing 22% wheat bran gave

higher digestibilities compared to the control diet, which was attributed to its low fibre content. *Aspergillis niger* might have also produced feruloyl esterase which is known to expedite plant cell wall digestion in animals (Topkas *et al*., 2007; Anson *et al*., 2009).

The organ-body weight ratios indicated that there was no significant difference among the various treatment groups. However, the slight increase observed in the liver-body weight of rats in groups 4, than the liver of group 2 rats, may not be unconnected with the inflammation of the liver. Orisakwe *et al.* (2003) reported that toxic effect of chemical or drug after administration could lead to that increase or decrease in weight of an organ.

Furthermore, the feeds were evaluated for their effects on marker enzymes in rat serum and tissues. The marker enzymes assayed included; ALP, ALT and AST. Increase in performance of these enzymes in the serum over that of the control, is usually an indication of enzyme leakage from the tissues into the serum, as a result of slight or major damage to the cell membranes of the tissues involved (liver, kidney and heart). The pretreated feeds caused inconsistent alterations to the ALP, ALT and AST levels in rat serum and tissues relative to the control. These data are consistent with previous reports by El-Ashry *et al.* (2001) and El-Shaer (2003). In rations supplemented with *A*. *niger* fermented products and fed to rats caused significant variations in levels of ALP, ALT and AST (El-Ashry *et al.,* 2001; El-Shaer, 2003). Perhaps, the alterations to the biochemical parameters may indicate a state of adaptation by the rats to the pretreated feeds.

Conclusion

Aspergillus sp. KM proved to be an excellent source of cellulase enzyme, which liberated the reducing sugar as a result of degradation of the substrate. The pre-treated rice and wheat bran was successfully bio-converted and incorporated as feed. Feed formulated with NH_3 pre-treated wheat bran gave the highest weight of the rats. Furthermore, no noticeable biochemical alterations have resulted from feeding the rats with the fermented wheat and rice bran. Fermented wheat bran has a potential to improve the protein value of the feed and increase the weight of animals. Further investigations are required to carry out the anti-nutritional factors, vitamins, minerals and other metabolites that could be liberated during the course of the fermentation.

Acknowledgements

We appreciate the supports of the laboratory technologists in the Departments of Biochemistry and Microbiology, Faculty of Life Sciences, University of Ilorin, Nigeria.

REFERENCES

Adney B. and Baker J. (2006). Measurement of cellulase activities In : Chemical Analysis and Testing Task Laboratory Analytical Procedure LAP 006. National Renewable Energy Laboratory (NREL)

Ahmadi AR, Ghoorchian H, Hajihosaini R and Khanifar J 2010 Determination of the amount of protein and amino acids extracted from the microbial protein (SCP) of lignocellulosic wastes. *Pakist. J. Biol. Sci*. **13** (8) 355-361.

Anson NM, Selinheimo E and Havenaar R 2009 "Bioprocessing of wheat bran improves *in vitro* bioaccessibility and colonic metabolism of phenolic compounds". *J. Agric. Food Chem*. **57** (14) 6148–6155.

A.O.A.C. 1990. Official methods of analysis Association of official analytical chemists 15th edition Washington DC.

Baig MMV, Baig MLB, Baig MIA and Yasmeen M 2004 Saccharification of banana agro-waste by cellulolytic enzymes. *Afric. J. Biotechnol.* **3** (9) 447-450.

Chinedu SN, Eni AO, Adeniyi AI and Ayangbemi JA 2010 Assessment of growth and cellulase production of wild-type microfungi isolated from Ota, Nigeria. *Asian J. Plant Sci.* **9** (3) 118-125.

De Vries RP and Visser J 2001 Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. *Microbiol. Molecul. Biol. Rev.* **65** (4) 479-522.

El-Ashry MA, Motagally AZ and Maareck YA 2001 Effect of *A. niger* with its culture on performance of rats. *Egypt J. Nutr. Feeds* **4** 605-606.

El-Shaer E K H I 2003 Effect of *A. niger* culture supplementation and concentrate roughage ratio on performance of growing rats. *Egypt. J. Nutr. Feeds* 6 200-206.

Hankin L and Anagnostaks ISL (1975) The use of solid media for detection of enzyme production by fungi. *Mycologia* **47** 597-607.

Islam MS, Khan M and Reza A 2009 Effect of wheat bran substitution for corn and dehydrated Alfalfa on finishing Lambs. *Banglad. J. Anim. Sci* **38** (1 & 2) 61-66

Jahromi MF, Liang JB, Rosfarizan M, Goh YM, Shokryazdan P and Ho YW 2010 Effects of *Aspergillus niger* (K8) on nutritive value of rice straw *Afric. J Biotechnol*. **9** (42) 7043-7047

Klich MA 2002 Identification of common *Aspergillus* species Utrecht, The Netherlands Central bureauvo or Schimmelcultures. **53** (4–5) 426–32.

Ko JK, Bak JS, Jung MW, Lee HJ, Cheol IG, Kim TH and Kim KH 2009 Ethanol production from rice straw using optimized aqueous ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Biores.Technol*. **100** (19) 4374.

Linde AC Lam JB and Morrison CG 2009 Acid and Alkaline pretreatment techniques *Adv. Microbiol. research.* **6** 54-78.

Louime C and Uckelmann H 2008 Cellulosic ethanol: securing the planet future energy needs. Inter. J. Molecul. Sci. **9** (5) 838-841.

Mendel M Hontz L and Nystrom J 1974 Enzymatic hydrolysis of waste cellulose. *Biotechnol. bioeng*. 16 (11) 1471-1493

Miller GL, Blum R, Glennon WE and Burton AL 1960 Measurement of carboxylmethyl cellulase activity. 1 *Aanlytic. Biochem*. 1(2) 127-132

Mtui G Nakamura Y 2005 Bioconversion of lignocellulosic waste from selected dumping sites in Dares Salaam, Tanzania. *Biodegrad.* **16** (6) 493-499.

Orisakwe OE Hussaini DC and Afonne OJ 2003 Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. *Reprod. Toxicol. 18* 295-298.

Pandy A 2003. Solid state fermentation. *Biochem. Eng. J.* 13 81-84.

Pandy A, Socool CR Rodriguez-leon JA and Nigam P 2001 Solid state fermentation in biotechnology 1st Edn. asiatech publishers Inc. (API), New delhi, ISBN: 81-87 680-06-7.

Pandy A Socool C.R and Mitchell D 2000 New developments in solid state fermentation in bioprocess and bioproducts. *Proc. Chem* **35** 1153-1169.

Preston R L 2006 Feed composition tables. www.Beefmagazine.com. (Access on December 13, 2013)

Rahnama N, Mamat S, MdShah U, Ling F H, AbdulRahman N and Ariff AB 2013 Effect of alkali pretreatment of rice straw on cellulase and xylanase production by local *Trichoderma harzianum* SNRS3 under solid state fermentation. *Bioresources.com*, **8** (2) 2881–2896.

Topakas E, Vafiadi C and Christakopoulos P 2007 Microbial production, characterization and applications of feruloyl esterases *Proc. Biochem*. **42** (4) 497–509.

Sun WC, Cheng CH and Lee WC 2008 Protein expression and enzymatic activity of cellulases produced by *Tichoderma reseei* Rut C-30 on rice straw Proc. biochem. **43** (10) 1083-1087.