The effect of using solid-state fermented peeled and unpeeled cassava root tubers and limiting amino acid supplementation on metabolisable energy for meat-type cockerels

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The effect of using solid-state fermented peeled and unpeeled cassava root tubers and limiting amino acid supplementation on metabolisable energy for meat-type cockerels

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A B S T R A C T

A preliminary *in-vitro* solid-state fermentation of peeled (PCRM) and unpeeled cassava root meal (UCRM) using *Aspergillus niger* was conducted followed by a force-feeding experiment to investigate the effect of processing, solid-state fermentation and limiting amino acid supplementation on metabolisable energy (ME) of peeled (PCRM) and unpeeled (UCRM) cassava root meal for meat-type cockerels. Forty eight, 84 d-old meat-type cockerels (Ross 308) were assigned to 8 treatments consisting of 6 birds per treatment laid out in a 2 × 2 × 2 factorial arrangement of treatment consisting of PCRM and UCRM subjected or not to solid-state fermentation and supplemented with and without limiting amino acids. Additional 6 cockerels were also used for endogenous study. Peeling of cassava root increased ($P < 0.05$) gross energy content of the resultant cassava meal when compared with UCRM. Solid-state fermentation using *Aspergillus niger* increased ($P < 0.05$) the crude ash, ether extract and arginine concentration of PCRM and UCRM. Solid-state fermented PCRM recorded the highest ($P < 0.05$) amylopectin, least ($P < 0.05$) resistant starch and hydrocyanide concentration. Highest ($P < 0.05$) apparent metabolisable energy (AME) and nitrogen corrected AME (AMEn) values were obtained for cockerels fed with solid-state fermented PCRM supplemented with or without amino acid. However, supplementation of solid-state fermented PCRM with amino acid resulted in highest ($P < 0.05$) true metabolisable energy (TME) and nitrogen corrected TME (TMEn) for meat-type cockerels. Reduced ($P < 0.05$) AME and AMEn values were recorded for UCRM, regardless of solid-state fermentation and amino acid supplementation. In conclusion, solid-state fermentation and amino acid supplementation of PCRM resulted in improved AME, AMEn, TME and TMEn values for meat-type cockerels. Amino acid supplementation had no improvement on AME, AMEn and TME values of UCRM for meat-type cockerels.

Keywords: Amino acid supplementation, Cassava root meal, Cockerels, Metabolisable energy, Solid-state fermentation

1. INTRODUCTION

Cassava (*Manihot esculenta*) root is a cheap and sustainable energy feedstuff with potential to replace most conventional cereal grains in the tropics (Oso et al., 2014). Cassava root is rich in digestible starch, gross energy content (El-sharkawy, 2012) and has been used to a limited extent in poultry nutrition (Eruvbetine et al., 2003; Oso et al., 2014). However, the presence of hydrocyanide (HCN) residues, reduced protein levels, poor protein quality and reduced concentration of sulphur containing amino acids in cassava root constituted the major constraints to its maximal utilization as energy feedstuffs in poultry nutrition (Banea-Mayambu et al., 1997). During cassava processing which convert cyanide to a less toxic thiocyanate, the enzyme 'rhodnase' contained in cassava root utilizes the constituent methionine and other sulphur containing amino acids as sulfur donor (Cardoso et al., 2005). Thus, sulphur amino acids become grossly deficient in cassava-based diets fed to poultry birds. Hence, to maximally harness the rich energy potential of cassava root in poultry nutrition, it is essential to supplement cassava root based diets with limiting amino acids.

Cassava peeling process is the removal of the topmost layer of cassava root prior utilization as food or feed. This processing methods helps to reduce the resultant hydrocyanide (HCN) content in cassava root product since the largest concentration of HCN in cassava root is located on the uppermost layer (Bruijn, 1973). Preliminary study showed improved growth performance

of broilers fed diet containing graded levels of peeled cassava root meal when compared with group fed diet containing unpeeled cassava root meal (Akapo et al., 2014).

Solid-state fermentation with fungal culture has been recognized as a means of nutritionally enriching and detoxifying few cassava products (Oboh and Akindahinsi, 2003). Filamentous fungi such as *Aspergillus niger* been widely used in food industries for commercial solid-state fermentation due to its ease of culturing and absence of pathogenic ability (Berka et al., 1992). *Aspergillus niger* has the capacity to produce extracellular enzymes (such as hemicellulases, hydrolases, pectinases, protease, amylase and lipases), degrade fibre and enrich its substrate (Mathivanan et al., 2006; Villena and Gutierrez-Cornea, 2007). The present study seeks to evaluate the effect of processing, solid-state fermentation and limiting amino acid supplementation on metabolisable energy of peeled and unpeeled cassava root meal for meat-type cockerels.

2. MATERIALS AND METHODS

2.1. Processing of cassava root

Freshly harvested cassava root tubers (TMS 30572) were washed with water and divided into two equal batches. One batch was manually chipped without prior peeling to obtain the whole cassava chips (WCC) while the other batch was peeled (removal of 0.5 cm uppermost thick layer) before chipping to yield the peeled cassava chips (PCC). Both WCC and PCC were dried (10–11 % moisture content) and milled (2.5 mm sieve) separately to yield the unpeeled (UCRM) and peeled cassava root meal (PCRM), respectively.

87 *2.2. Solid-state fermentation of cassava root meal*

88 Pure laboratory strain of *Aspergillus niger* (Chinese International Centre for Type Culture
89 Collection; CICC, No. 41126) was used as inoculum. A total of 8 kg cassava meal (consisting of
90 4 kg UCRM and 4 kg PCRM) were measured and used for this study. Twenty (20) sub-samples
91 of UCRM and PCRM, each weighing 200 g were measured and placed into separate conical
92 flasks. Thus, forty (40) conical flasks were used in all for the study (20 flasks for UCRM and 20
93 flasks for PCRM group). All UCRM and PCRM samples contained in flasks were randomly
94 assigned, each into 2 treatments consisting of solid-state fermented and unfermented group. Thus
95 there were four treatments in all laid out in a 2 × 2 factorial arrangement of peeled (PCRM) and
96 unpeeled (UCRM) cassava root meal, each subjected or not to solid-state fermentation. Samples
97 (contained in flasks) subjected to solid-state fermentation were moistened (250 g/kg Moisture
98 content) each with nutrient solution (containing analytical grade of 80 g urea, 7 g $MgSO_4.2H_2O$,
99 13 g KH_2PO_4 and 20 g citric acid) and inoculated with 2×10^7 fungal spore of *A. niger* per gram
100 of sample. Each conical flask was air-sealed and the substrate incubated (30^o C) for 6 days in a
101 bed-packed incubator. At the end of incubation period, fermented samples (contained in each
102 flask) were sterilized (120^o C for 20 min) and used for subsequent chemical analysis.

103 *2.3 Chemical analysis of samples*

104 Fermented samples of UCRM (*n = 10*) and PCRM (*n = 10*) and respective unfermented samples
105 were analyzed for dry matter (DM) by drying at 80^{o}C for 24 h (AOAC; 925.10). Ash was
106 measured in a muffle furnace (510^o C for 18 h), crude protein (6.25 × N) was determined by
107 LECO FP-200 Analyser (St Joseph, MI, USA), oil was extracted with petroleum spirit using the
108 soxhlet method (AOAC, 1990). Gross energy (Adiabatic bomb calorimeter, Model 1261; Parr

Instrument Co., Moline, IL, USA), fibre fraction (Van Soest et al., 1991), tannin (Makkar et al., 1993) and hydrocyanide content (De Bruijn, 1971) of samples were determined following standard procedures. The amylopectin (Amylose/Amylopectin kit, Megazyme International Co. Ireland) and resistant starch content (KRSTAR 08/11 Test kit, Megazyme International Co. Ireland) of samples were determined using appropriate commercial kits. Mineral analysis (ICP–MS, Agilent 7500 cx, Agilent Technologies) and amino acid analysis (RP-HPLC; Agilent 1100, Palo Alto, CA, USA) of the samples were also determined. All laboratory analysis was done at the Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan Province, China.

2.4 Metabolisable energy determination using gavage method

The experimental protocol used in this study was approved by the Institutional Animal Care and Welfare Committee of the Institute of Subtropical Agriculture (ISA), Chinese Academy of Sciences, P.P.R China (Approval No. ISA AEC 2013-014). A total of fifty four (54) meat-type cockerels (Ross 308, 12-weeks-old) of average weight 2250g $\pm$ 115 were used in all for this experiment. Forty eight (48) cockerels were assigned to 8 treatments in a 2 × 2 × 2 factorial arrangement of treatment consisted of peeled (PCRM) and unpeeled (UCRM) cassava root meals, fermented or not with *A. niger* and supplemented with and without limiting amino acids. There were 6 replicates per treatment of 1 bird per replicate. The remaining 6 cockerels were used for endogenous study. Birds were kept in individual iron-type battery cages (each of dimension 35 × 35 × 50; LBH) and fed commercial diets prior the commencement of the experiment. The amino acids supplemented were as follows: L-lysine (0.75 g/100 g cassava meal), DL-methionine (1.5 0 g/100 g cassava meal), L-arginine (0.75 g/100 g cassava meal) and L-cysteine (0.75 g/100g cassava meal). Birds were orally gavaged 30 g of respective processed

cassava meal after 48 hr of starvation following the standard procedure outlined by Mc Nab and Blair (1988). All birds had free access to drinking water while birds assigned to endogenous group were dosed each with warm glucose solution (30 g of glucose/50 ml of warm water). Excreta voided from each bird following the feeding procedure were collected quantitatively. All the birds survived the experiment as no mortality was recorded throughout the study. Gross energy of samples of excreta was measured while the following equations were used to calculate apparent metabolisable energy (AME), nitrogen corrected apparent metabolisable energy (AMEn), true metabolisable energy (TME), and nitrogen corrected true metabolisable energy (TMEn) of test ingredient (Sibbald, 1989):

$$\text{AME /g of feed} = [(\text{Fi} \times \text{GEf}) - (E \times \text{GEe})]/\text{Fi}$$

Where Fi is the feed intake (g on dry matter basis), *E* is quantity of excreta output (g on dry matter basis), GEf is the gross energy (MJ/ kg) of feed, and is GEe the gross energy (MJ/ kg) of excreta.

$$\text{AMEn /g of feed} = \frac{\{[(\text{Fi} \times \text{GEf}) - (E \times \text{GEe})] - (\text{NR} \times 36.5)\}}{\text{Fi}}$$

where nitrogen retention (NR) = (Fi × Nf) – (*E* × Ne), Nf is the nitrogen content (g/kg) of feed, Ne is the nitrogen content (g/kg) of excreta.

$$\text{TME /g of feed} = \frac{\{[(\text{Fi} \times \text{GEf}) - (E \times \text{GEe})] + (\text{FEm} + \text{UEe})\}}{\text{Fi}}$$

where FEm is metabolic faecal energy (kJ) (calculated from gross energy of excreta from endogenous loss), and UEe is endogenous urinary energy (kJ) (This is assumed zero since urine and faeces are passed together).

$$\text{TMEn /g of feed} = \frac{\{[(Fi \times GEf) - (E \times GEe)] - (NR \times K)\} + \{(FEm + UEe) + (NRo \times 36.5)\}}{Fi}$$

Where NR and NRo are estimates of nitrogen retention for fed (experimental) and starved (control) birds, respectively.

2.5 Statistical Analysis

As regards data obtained from compositional chemical analysis of unfermented and solid-state fermented UCRM and PCRM, replicate units in conical flasks ($n = 10$ per treatment) served as experimental units for statistical analysis. These data was analysed as a two factor model (cassava peeling × solid-state fermentation) consisting of peeled and unpeeled cassava root, subjected or not to solid-state fermentation. For the analysis of data obtained from estimation of metabolisable energy using gavage method, individual bird was used as the experimental unit ($n = 6$ per treatment). Data obtained from gavage studies were analysed as a three factor model (cassava peeling × solid-state fermentation × amino acid supplementation) consisting of peeled and unpeeled cassava root, subjected or not to solid-state fermentation and supplemented with or without amino acids. All data generated in this study were subjected to analysis of variance using the general linear models procedure of the SAS (SAS Institute, 2002) to determine the main effects and their respective interactions. Significant differences were considered at $P < 0.05$.

2.6 Statistical Model

For two factor model (cassava peeling × solid-state fermentation) analysis of chemical composition of peeled and unpeeled cassava root, the model used is as follows:

$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk}$

Where Y_{ij} = Observed value of the dependent variable

μ = Population mean

A_i = Main effect of cassava peeling (peeled, unpeeled)

B_j = Main effect of solid state fermentation (fermented, unfermented)

AB_{ij} = Interraction effect of cassava peeling and solid state fermentation

ε_{ijk} = Random residual error.

For three factor model (cassava peeling × solid-state fermentation× amino acid supplementation) analysis of metabolisable energy determination of peeled and unpeeled cassava root, the model used is as follows:

$Y_{ij} = \mu + A_i + B_j + C_k + AB_{ij} + BC_{jk} + AC_{ik} + ABC_{ijk} + \varepsilon_{ijkl}$

Where Y_{ij} = Observed value of the dependent variable

μ = Population mean

A_i = Main effect of cassava peeling

B_j = Main effect of solid state fermentation

C_k = Main effect of amino acid supplementation

AB_{ij} = Interraction effect of cassava peeling and solid state fermentation

BC_{jk} = Interraction effect of solid state fermentation and amino acid supplementation

AC_{ik} = Interraction effect of cassava peeling and amino acid supplementation

ABC_{ijk} = Interraction effect of cassava peeling, solid state fermentation and amino acid supplementation

ε_{ijkl} = Random residual error

3. RESULTS AND DISCUSSION

3.1. Solid-state fermentation of peeled (PCRM) and unpeeled (UCRM) cassava root meal

Solid-state fermentation of PCRM and UCRM with *A. niger* resulted in increased ($P < 0.05$) ether extract, crude ash and reduced ($P < 0.05$) dry matter content (Table 1). Increased ether extract content of resultant meal (UCRM and PCRM) following solid-state fermentation could be attributed to the ability of *A. niger* to synthesize long chain fatty acids from acetyl co-enzymes A and other complex unsaturated lipids during fermentation (Iyayi and Aderolu, 2004). Increased ash content recorded for fermented UCRM and PCRM when compared with unfermented meal could be due to increased available mineral caused by metabolic activities of the fermenting organism. The highest ($P < 0.05$) ash content obtained for fermented UCRM could be attributed to the rich mineral content of the outer cassava peel contained in UCRM coupled with the fermentation. The outer layer of cassava root (peel) has been reported to contain richer macro-minerals than the pulp (Akapo et al., 2014).

Peeling of cassava root subjected or not to solid-state fermentation using *A. niger* resulted in improved (Cassava processing × Solid-state fermentation, $P < 0.05$) gross energy content and reduced ($P < 0.05$) hydrocyanide content (HCN) of the resultant meal when compared with the unpeeled cassava meal. UCRM contain fibrous outer peels which could lead to a dilution effect of the constituent energy hence reduced energy content. Cassava root peeling led to reduced HCN because the highest concentration of HCN in cassava root is located on the outer peel when compared with the inside pulp (Bruijn 1973). Hence, peeling of cassava root to yield UCRM will yield a product with reduced HCN content.

Solid-state fermentation of PCRM using *A. niger* resulted in a fermented product with reduced NDF ($P < 0.01$) and ADF ($P < 0.05$) content. Fermentation with *A. niger* thus resulted

221 in efficient breakdown of the constituent fibre. *A. niger* has been earlier reported to produce
222 ligno-cellulolytic enzymes during fermentation which break down constituent fibre in cassava
223 root (Mathivanan et al., 2006; Villena and Gutierrez-Cornea, 2007). Solid-state fermentation of
224 PCRM in the current study also showed reduced ($P < 0.05$) resistant starch content and improved
225 ($P < 0.05$) amylopectin content suitable for products that required adhesion (Bergmann et al.,
226 1988). *A. niger* has been reported to degrade starch granules for substrate enrichment (Soccol et
227 al., 1994).

228 Solid-state fermentation of both PCRM and UCRM showed reduced ($P < 0.05$) Cu levels
229 of the resultant fermented products. In fact, solid-state fermentation of PCRM resulted in
230 reduced (Cassava processing × Solid-state fermentation, $P < 0.05$) K and Zn content of the
231 fermented cassava products (Table 1). The effect of solid-state fermentation on mineral profile of
232 cassava products has not been extensively investigated in literatures. The reduced concentration
233 of Cu noticed for solid-state fermented PCRM and UCRM could be due to the adsorption ability
234 of the fungi. *A. niger* is known to produce large quantities of organic acids such as citrate and
235 gluconate, both of which are capable of leaching or precipitating metals out of a number of
236 substrate by either adsorption to fungal cell wall components, or complexation of the metals
237 (Bosshard et al., 1996).

238 Amino acid profile of PCRM and UCRM subjected or not to solid-state fermentation is as
239 shown in Table 2. Solid-state fermentation of PCRM and UCRM increased ($P < 0.05$) the
240 arginine concentration of the resultant fermented products. The improved arginine concentration
241 obtained in fermented PCRM and UCRM when compared with the unfermented meals
242 corroborated the earlier findings that fungal fermentation of cassava products improved the

243 resultant amino acid profile (Oboh and Akindahinsi, 2003). Arginine is noted for its role in
244 protein synthesis and its consequence influence on growth of animals (Kidd et al., 2001).
245
246 *3.2. Metabolisable energy determination of PCRM and UCRM using gavage method*
247 Metabolisable energy values of PCRM and UCRM subjected or not to solid-state fermentation
248 and supplemented with and without amino acids is as shown in Table 3. Solid-state fermentation
249 of PCRM supplemented or not with amino acid recorded the highest (Cassava processing ×
250 Solid-state fermentation × amino acid supplementation, $P < 0.05$) AME and AMEn for meat-
251 type cockerels. Highest AME and AMEn values of fermented PCRM recorded in this study
252 regardless of amino acid supplementation could be due to improved gross energy content and
253 reduced HCN content of PCRM following cassava root peeling and solid state-fermentation.
254 This improved AME and AMEn of fermented PCRM could also be linked with the increased oil
255 content produced by *A. niger* during solid-state fermentation (Iyayi and Aderolu, 2004).
256 Mathivanan et al. (2006) reported that solid-state fermentation produce digestive enzymes which
257 pre-digest substrates and thus foster increased nutrient availability, digestibility and energy
258 metabolisability.
259 Reduced (Cassava processing × Solid-state fermentation × amino acid supplementation,
260 $P < 0.05$) AME and AMEn values of UCRM (regardless of solid-state fermentation and amino
261 acid supplementation) obtained in the present study for meat-type cockerels could be linked with
262 high fibrous constituent of UCRM. Fibrous feedstuffs have been reported to reduce energy
263 metabolisabilty of poultry birds (Janssen and Carré, 1985). Meanwhile, peeling of the outer
264 layer of cassava root helps in reducing the constituent fibre and thus leads to increased available

energy of the resultant product (PCRM). Amino acid supplementation showed no positive contribution to AME and AMEn values of UCRM from this study.

Highest (Cassava processing × Solid-state fermentation × amino acid supplementation, $P < 0.05$) TME and TMEn values obtained for fermented and amino acid-supplemented PCRM obtained for meat-type cockerels in the present study underscores the importance of cassava peeling process, solid-state fermentation and amino acid supplementation in improving the TME and TMEn values of PCRM. However, amino acid supplementation showed no improvement on TME and TMEn values of unfermented UCRM. Although, slight improvement on TMEn values of UCRM was noticed following solid-state fermentation, however these TMEn values were lower than corresponding values obtained for cockerels fed with fermented and amino acid-supplemented PCRM.

4. CONCLUSION

The present study provides background information on the possible utilization of peeled and unpeeled cassava root as energy feedstuffs in the nutrition of meat-type cockerels. It was concluded that solid-state fermentation and amino acid supplementation of peeled cassava root meal had the best metabolisable energy values (AME, AMEn, TME and TMEn) for meat-type cockerels. Although solid-state fermentation of unpeeled cassava root meal had little prospect for improved TMEn, amino acid supplementation of unpeeled cassava root meal had no improvement on AME and AMEn values for meat-type cockerels.

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5. **REFERENCES**

Akapo, O.A., Oso, A. O., Bamgbose, A.M., Sanwo, K.A., Jegede, A.V., Sobayo, R.A., Idowu, O.M.O., Juexin Fan; Lili Li; Olorunsola, R.A., 2014. Effect of cassava (*Manihot esculenta* Crantz) root peeling and inclusion levels on growth performance, hydrocyanide intake, haematological indices and serum thiocyanate concentration of broiler chicks. Trop. Anim. Health Prod. 46, 1167-1172

A.O.A.C., 1990. The official method of Analysis, Association of official analysis chemist, 13th Edition, AOAC International, Washington DC

Banea-Mayambu, J. P., Tylleskar, T., Gitebo, N., Matadi, N., Gebre-Medhin, M., Rosling, H., 1997. Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neuron disease konzo in former Zaire. Trop. Med. Intl. Health. 2, 1143- 1151

Bergmann, F.W., Abe, J. I., Hizukuri, S., 1988. Selection of microorganisms which produce raw starch-degrading enzymes. Appl. Microbiol. Biotechnol. 27**,** 443-446.

Berka, R.M., Dunn-Coleman, N., Ward, M., 1992. Industrial enzymes from Aspergillus species. In. Bennett, J.W., Klich, M.A. (Eds.), Aspergillus. Biology and Industrial Applications. Butterworth- Heinemann, Boston, pp. 155-202.

308 Bosshard, P., Bachofen, R., Brandl, H., 1996. Metal leaching of fly ash from municipal waste
309 incineration by *Aspergillus niger*. Environ. Sci. Technol. 30**,** 3066-3070.

310 Bruijn, G.H., 1973. The cyanogenic character of cassava. In. Nestel, B., MacIntyre R.
311 (Eds).Workshop on chronic cassava toxicity and useas livestock feeds, Ottawa,
312 International Development Research Centre (IDRC-10e), pp. 43-48.

313 De Bruijn, G. H., 1971. A study of the cyanogenic character of cassava. *Meded.*
314 *Lanbouwhogesch.* Wageningen **71,** 1–140.

315 Cardoso, A.P., Mirione, E., Ernesto, M., Massaza, F., Cliff, J., Haque, M.R., 2005. Processing of
316 cassava roots to remove cyanogens. J. Food Comp. Anal. 18, 451 –460.

317 El-Sharkawy, M., 2012. "Stress - Tolerant Cassava. The Role of Integrative Ecophysiology -
318 Breeding Research in Crop Improvement. Open J. Soil Sci. 2**,** 162-186.

319 Eruvbetine, D., Tajudeen, I.D., Adeosun, A.T., Olojede, A.A., 2003. Cassava leaf and tuber
320 concentrate in diet for broiler chickens. Bio-resource Technol. 86**,** 277 –281

321 Janssen, W.M., Carre, B., 1985. Influence of fibre on digestibility of poultry feeds, In. Haresign,
322 W. and D. J. A. COLE (Eds) Recent Advances in Animal Nutrition, London,
323 Butterworths. pp. 71-86.

324 Iyayi, E. A., Aderolu, Z. A., 2004. Enhancement of the feeding value of some agro-industrial by
325 products for laying hens after their solid state fermentation with Trichoderma viride. Afr.
326 J. Biotechnol. 3, 182–185

327 Makkar, H. P. S., Blummel, M., Bowwy, N. K., Becken, K., 1993. Determination of tannins and
328 their correlation with chemical and protein precipitation method, J. Sci. Food Agric. 61,
329 161–185.

330 Mathivanan, R., Selvaraj, P., Nanjappan, K., 2006. Feeding of fermented soybean meal on
331 broiler performance. Intl. J. Poult. Sci. 5**,** 868 –872.

332 Mc Nab, J.M., Blair, J.C., 1988. Modified assay for true and apparent metabolisable energy
333 based on tube-feeding. Brit. Poult. Sci. 29, 697–707.

334 Oboh, G., Akindahunsi, A.A., 2003. Biochemical Changes in Cassava Products (flour and
335 gari) subjected to *Saccharomyces cerevisae* solid media fermentation. Food Chem.
336 82, 599–602.

337 Oso, A. O., Akapo, O., Sanwo, K.A., Bamgbose, A.M., 2014. Utilization of unpeeled cassava
338 (*Manihot esculenta* Crantz) root meal supplemented with or without charcoal by broiler
339 chickens. J. Anim. Physiol. Anim. Nutri. 98**,** 431-438.

340 SAS INC., 2002. SAS® User's Guide. Statistics, Release 8.02. SAS Inc., Cary, NC, USA.

341 Sibbald, I.R., 1989. Metabolisable energy evaluation of poultry diets. In. Cole, D.J.A., Haresign,
342 W. (Eds.), Recent Developments in Poultry Nutrition. Anchor Press Ltd., Tiptree, Essex,
343 pp. 12– 26.

344 Soccol, C.R., Martin, B., Raimbault, M., Lebeault, J.M., 1994. Breeding and growth of
345 Rhizopus in raw cassava by solid state fermentation. Appl. Microbiol. Biotechnol. 41,
346 330-336

347 Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent
348 fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74**,**
349 3583–3597.

Villena, G.K., Gutierrez-Correa, M., 2007. Production of lignocellulolytic enzymes by *Aspergillus niger* bio-films at variable water activities. J. Biotechnol. 10, 124–140.

368 **Table 1**. Effect of solid-state fermentation on the chemical composition and energy content of

369 unpeeled and peeled cassava root meal

Cassava root processing (CRP)	Unpeeled		Peeled		Pooled SEM	*Level of significance*		
Solid-state fermentation (SSF)	No	Yes	No	Yes		CRP	SSF	CRP × SSF
Measurements								
Dry matter (g/kg)	907.1[a]	719.2[b]	910.25[a]	722.50[b]	40.22	NS	<0.05	<0.05
Crude ash (g/kg)	11.4[c]	15.1[a]	10.9[c]	13.2[b]	4.00	<0.05	<0.01	<0.05
Ether extract (g/kg)	12.5[b]	17.5[a]	12.2[b]	18.3[a]	3.99	NS	<0.05	<0.05
Crude protein (g/kg)	14.5	15.0	14.1	15.5	0.12	NS	NS	NS
Gross energy (MJ/kg)	14.10[b]	14.21[b]	16.98[a]	17.25[a]	4.02	<0.05	NS	<0.05
NDF (g/kg)	360.5[a]	330.0[b]	320.7[c]	305.2[d]	36.44	<0.01	<0.01	<0.01
ADF (g/kg)	250.2[a]	227.5[b]	225.7[b]	200.7[c]	32.55	<0.05	<0.05	<0.05
Amylopectin (g/kg)	809[c]	834.9[b]	830.9[b]	874.5[a]	72.10	<0.05	<0.05	<0.05
Resistant starch (g/kg)	98.50[a]	48.0[c]	70.50[b]	35.0[d]	12.75	<0.05	<0.05	<0.05
hydrocyanide (mg/kg)	30.4[a]	30.20[a]	23.6[b]	22.50[b]	4.74	<0.05	NS	<0.05
Tannin (%)	0.32	0.30	0.30	0.29	0.02	NS	NS	NS
Ca (mg/kg)	0.31	0.29	0.30	0.30	0.001	NS	NS	NS
P (mg/kg)	0.52	0.50	0.51	0.54	0.055	NS	NS	NS
Mg (mg/kg)	0.62	0.59	0.60	0.60	0.082	NS	NS	NS
Mn (mg/kg)	0.009	0.008	0.009	0.009	0.0001	NS	NS	NS
Cu (mg/kg)	0.009[a]	0.003[b]	0.009[a]	0.002[b]	0.0007	NS	<0.05	<0.05
Fe (mg/kg)	0.11	0.10	0.11	0.10	0.004	NS	NS	NS
K (mg/kg)	6.2[a]	5.70[a]	6.4[a]	5.0[b]	0.92	NS	<0.05	<0.05
Zn (mg/kg)	0.03[a]	0.02[a]	0.03[a]	0.01[b]	0.007	NS	<0.05	<0.05

370 [a, b] Mean with different superscripts in each row are significantly different (P<0.05)

371 **Table 2**. Effect of solid-state fermentation on amino acid profile of unpeeled and peeled cassava

372 root meal

Cassava root processing (CRP)	Unpeeled		Peeled		Pooled SEM	*Level of significance*		
Solid-state fermentation (SSF)	No	Yes	No	Yes		CRP	SSF	CRP × SSF
Measurements (*g/100g protein*)								
Asparagine	0.14	0.15	0.15	0.14	0.002	NS	NS	NS
Threonine	0.04	0.05	0.05	0.04	0.002	NS	NS	NS
Serine	0.07	0.07	0.08	0.07	0.003	NS	NS	NS
Glutamine	0.40	0.42	0.41	0.40	0.001	NS	NS	NS
Glycine	0.10	0.10	0.11	0.10	0.003	NS	NS	NS
Alanine	0.14	0.14	0.12	0.14	0.005	NS	NS	NS
Cysteine	0.04	0.05	0.05	0.04	0.010	NS	NS	NS
Valine	0.09	0.10	0.09	0.10	0.02	NS	NS	NS
Methionine	0.01	0.02	0.10	0.10	0.002	NS	NS	NS
Isoleucine	0.05	0.06	0.05	0.06	0.001	NS	NS	NS
Leucine	0.15	0.15	0.15	0.16	0.001	NS	NS	NS
Tyrosine	0.04	0.05	0.05	0.05	0.002	NS	NS	NS
Phenylalanine	0.07	0.06	0.06	0.07	0.001	NS	NS	NS
Lysine	0.02	0.01	0.01	0.01	0.002	NS	NS	NS
Histidine	0.03	0.03	0.02	0.02	0.001	NS	NS	NS
Arginine	0.08[b]	0.15[a]	0.09 [b]	0.17 [a]	0.012	NS	<0.05	<0.05
Proline	0.15	0.16	0.15	0.16	0.004	NS	NS	NS

373 [a, b] Mean with different superscripts in each row are significantly different (P<0.05)

374 NS= Not significant

375 **Table 3.** Metabolisable energy values of peeled and unpeeled cassava root meal subjected to
376 solid-state fermentation and supplemented with or without amino acids for meat-type cockerels

Attributes			AME	AMEn	TME	TMEn
Cassava root processing	Solid-state fermentation	Amino acid supplementation				
Unpeeled	No	No	11.62^{b}	11.85^{b}	12.01^{c}	12.33^{c}
Unpeeled	No	Yes	11.70^{b}	11.90^{b}	12.15^{c}	12.44^{c}
Unpeeled	Yes	No	11.92^{b}	12.20^{b}	12.42^{bc}	12.64^{b}
Unpeeled	Yes	Yes	11.99^{b}	12.35^{b}	12.50^{bc}	12.80^{b}
Peeled	No	No	12.22^{b}	12.32^{b}	12.51^{bc}	12.59^{b}
Peeled	No	Yes	12.10^{b}	12.15^{b}	12.49^{bc}	12.60^{b}
Peeled	Yes	No	12.85^{a}	12.90^{a}	13.29^{b}	13.34^{b}
Peeled	Yes	Yes	12.75^{a}	12.84^{a}	13.67^{a}	13.76^{a}
Pooled SEM			2.22	2.07	2.10	2.05
Significance						
Cassava root peeling			NS	<0.05	<0.05	NS
Solid state fermentation			NS	<0.05	<0.05	NS
Amino acid supplementation			NS	NS	NS	NS
Cassava root peeling × Solid state fermentation			<0.05	<0.05	<0.05	<0.01
Cassava root peeling × Amino acid supplementation			NS	<0.05	<0.05	<0.05
Solid state fermentation × Amino acid supplementation			NS	<0.05	<0.01	<0.05
Cassava root peeling × Solid state fermentation × Amino acid supplementation			<0.05	<0.05	<0.05	<0.05

377 [a,b,c,d] Values in the same column not sharing a common superscript are significantly different at *P*

378 *<0.05*.

379 NS= Not significant