PAT June, 2017; 13 (1): 30-35 ISSN: 0794-5213

Online copy available at www.patnsukjournal.net/currentissue

Publication of Nasarawa State University, Keffi

Fungus (*Aspergillus niger*) Treated Ackee Apple Seed Meal: Assay of Nutritive Value, Voluntary Intake and Digestibility in Pregnant Goat

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Abstract

This study was conducted to assess the nutritive value of Aspergillus niger treated Ackee apple seed meal (AASM) using sixteen (16) pregnant goats in a completely randomized design model. The goats were randomly grouped into four, with each group assigned different experimental diets. The experimental diets consisted of A (control) without the inclusion of fungus treated AASM while other diets had the inclusion of fungus treated AASM to replace groundnut cake (GNC) at 2.5%, 5.0% and 7.5% representing B, C and D respectively. Feed intake was monitored along with digestibility evaluation of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and ash. The results showed that fungus treated diets increased the proximate value of all the nutrients considered except CF. Crude protein, crude fat and ash contents increased respectively by 2.73, 2.55 and 0.41%. The animals placed on diet B had the highest (989.57g/day) DM intake, CF (198.70g/day) and EE (107.79g/day). Significantly higher (P<0.05) digestible DM, CF and EE was also observed for diet B. Apart from the digestibility coefficient for CP where diet B had the highest value (77.48%), diet D was relatively higher (83.11%) in DM intake, CF (76.53%) and EE (91.37%). It is therefore concluded that pregnant West African dwarf goats may effectively tolerate up to 7.5% inclusion of A. niger treated AASM without any ill-health.

Keywords: Anti-nutrition; Crude protein; Detoxification; Dwarf goat.

Introduction

One of the major environmental impediments to the realization of full genetic potential of indigenous livestock in Nigeria is nutrition. A poorly fed livestock has a greater tendency of poor growth, disease incidences and reduced productivity. Seasonal availability of forages and increasing competition for conventional and nutrient dense feedstuffs between livestock and man have also compounded the profitability of livestock enterprise. This has necessitated research emphasis on the utilization of non-competitive agricultural waste and by-products as well as other abundantly available non-conventional materials as feed resource. However, most of the non-conventional feed resources, particularly of plant origin, are faced with challenges of nutrient availability due to binding effects of anti-nutritional compounds (Gilani *et al*., 2012).

Ackee apple is an evergreen tree cultivated in the tropical and subtropical regions of the world, and produces abundant seed whose utilization as feed resource is limited by the presence of hypoglycine A and B and some other phytochemicals (Annougu *et al*., 2012; Dossou *et al.,* 2014). Inhibition of enzymatic processes leading to poor digestion in the digestive tract (Dolan, 2010) is a major problem associated with the consumption of the Ackee apple seed (AAS). A disease popularly referred to as "Jamaica vomiting sickness" was reported to be a systemic reaction after ingestion of AAS by man while hypoglycemia was another side effect of consuming AAS fruits (Barceloux, 2009; Grunes *et al.,* 2012). Thus, the need to detoxify the seed is imperative before utilization as a feed resource in ruminant feeding.

Various researchers have attempted detoxifying the AAS seed using chemical treatment (Annougu *et al.,* 2012). This may however pose some challenges on the receiving animal due to residual effect on the possible ionic compounds on the substrate. Biological detoxification using fungi has also been attempted and observed with improved result on livestock (Belewu *et al.,* 2015). However, physiological state of the animal may to some extent influence the utilization of fungi treated AAS and more especially in pregnant animals which have more sensitivity to nature of diet. This research was therefore aimed at assessing dietary inclusion of *Aspergillus niger* treated AAS on intake and digestibility of West African Dwarf pregnant goats.

The feeding trial of this research was conducted at the Teaching and Research Farm of the University of Ilorin while the laboratory analysis and Ackee Apple Seed treatment using fungi was carried out at the Animal Production Laboratory, University of Ilorin, Ilorin.

Sources of Ackee apple seed and Inoculum (fungus)

A reasonable quantity of fresh AAS was sourced from various villages surrounding University of Ilorin while the cultured fungus (*Aspergillus niger*) used for the seed treatment was collected from the Department of Microbiology, University of Ilorin.

Preparation of Substrate: The moisture content of the collected AAS was reduced using oven (WTC Binder, Model E-53, Germany) for a period of 72 hours at a temperature range of 60 – 70°C. The dried seed was subjected to particle size reduction using milling machine and sieved (mesh 25) in order to obtain ackee apple seed meal that was used as substrate in this study.

Preparation of Growth medium for the *Aspergillus niger*: A small quantity (10g) of Potato Dextrose Agar (PDA) and 250ml of distilled water were mixed in a conical flask which was later corked using cotton wool and the edge wrapped with aluminum foil. The mixture was transformed into homogenous suspension after rigorous shaking and slight application of heat via Bunsen burner for 2 – 3 minutes. Carbonation of the suspension was prevented during heat application by intermittent shaking. The homogenous mixture was sterilized using autoclave operated at 121°C for 15 minutes. The conical flask was removed and allowed to cool for a period of 5 – 10 minutes after which the content formed a clear yellow gelatinous solution of PDA. The PDA was poured into petri dishes and allowed to be solidified.

Inoculation and Incubation of Growth Medium and Substrate: A small quantity of *A. niger* was used to inoculate the freshly prepared sterilized PDA in petri dishes using sterilized wire loop. The inoculated medium was covered, cello-taped and allowed to incubate for a period of 72 hours at room temperature. This resulted into a massive growth of *A. niger* which was subsequently used to inoculate the substrate (Milled Ackee apple seed) after its sterilization in black polythene bags placed inside autoclave. This inoculation was carefully done to ensure even distribution of *A.* ***niger*** on thin layer of the substrate in a big bowl. The bowl was covered with polythene and placed in the laboratory for incubation within a period of 11days. The bowl was opened for proper mixing of content at the 7th day and final mixing at the 11th day. The content of the bowl was later oven dried at 70°C for 72 hours. The dry sample was included in the experimental diets shown in Table 1.

Chemical analysis: Proximate composition of the treated and untreated AASM, experimental diets and the faeces was done according to AOAC (1990).

Table 1: Composition of experimental diets containing different inclusion levels (%) of ***A. niger*** treated Ackee apple seed meal.

	Experimental Diets			
Feed Ingredients	A	B	C	D
Cassava peel (%)	53.00	53.00	53.00	53.00
Rice husk (%)	35.00	35.00	35.00	35.00
Groundnut cake (%)	10.00	7.50	5.00	2.50
A.niger treated AASM (%)	0.00	2.50	5.00	7.50
Salt (%)	1.00	1.00	1.00	1.00
Vitamin premix (%)	1.00	1.00	1.00	1.00

Experimental Animals: A total of sixteen (16) pregnant (naturally bred) does with an average weight of 25kg were randomly allocated to the four experimental diets (4 does per treatment) in a completely randomized design. The does (at their 2 – 3 month pregnancy stage) were acclimatized for a period of four weeks in metabolic cages during which *ad-libitum* supply of feed and water was ensured.

Data collection and statistical analysis: Feed intake of the goats was monitored throughout the period of feeding trial that lasted for six weeks. Faecal samples were collected during the last two weeks of the experiment. Data collected were subjected to analysis of variance while the mean separation was done using Duncan as specified by Minitab (2003).

Results and Discussion

The result of the proximate composition of untreated and treated AASM is indicated in Table 2. It was shown that ***A. niger*** treatment increased the nutritive value of all the parameters considered except crude fibre. Crude protein, crude fat and ash contents of the raw seed respectively increased by 2.73, 2.55 and 0.41% after subjecting it to fungus treatment. The dry matter content of treated AAS was also higher than that of raw seed with 2.50% while the crude fibre decreased by 0.54%. Increase in all the nutrients apart from fibre after the fungus treatment of AAS indicated an enrichment in the nutritive value. Enzymes, the major secretion from fungus after its proliferation on the substrate, helps in the pre-digestion of the substrate - AASM seed meal (Fox and Howlett, 2008). This invariably implies that some levels of fibre degradation took place on the raw AAS as a result of fungus treatment, and that the binding effect of hypoglycine A and B in the raw AAS (Hassall and Reyel (1995) was drastically reduced leading to increased nutrient availability. This affirmed the earlier report (Belewu and Akande, 2010) that elimination or reduction in the anti-nutritional compounds of some potential feedstuffs of plant origin can be facilitated using fungi treatment.

Table 2: Proximate composition of untreated and treated Ackee apple seed meal.

	Nutrient content (%)				
	Crude Protein	Crude fat	Crude fibre	Dry matter	Ash
Untreated ackee apple seed	15.65	12.20	14.15	91.90	4.03
Treated ackee apple seed	18.38	15.75	13.61	93.40	4.44

Table 3 shows the proximate composition of the experimental diets. The crude protein content of the diets ranged from 8.91 – 12.30%. The CP content decreased as inclusion levels of the fungus treated AASM increased. However, all the diets have CP higher than the minimum CP required (7%) by goat (NRC, 1981). Contrarily, the CF increased as the inclusion levels of the fungus treated AASM increased in the diets. The ether extract and the ash contents did not follow any trend.

Table 3: Proximate Composition of experimental diets

	Experimental Diets			
Nutrients (%)	A	B	C	D
Dry matter	93.65	92.30	93.00	93.60
Crude protein	12.30	10.30	10.28	8.91
Crude fibre	17.79	18.45	18.97	19.65
Ether extract	10.77	10.15	10.00	10.53
Ash	10.98	10.56	11.10	11.07

Average feed intake of the experimental diets during the feeding trial is indicated in Table 4. There was a significant difference ($P < 0.05$) among the experimental diets with respect to nutrient intake (dry matter, crude protein, crude fibre, ether extract and ash). Animals placed on diet B containing 0.25% fungus treated AASM had the highest intake across all the parameters investigated except crude protein. The highest dry matter intake (989.57g/day) recorded for the goats placed on diet B was significantly higher (P< 0.05) than the DM intake of other diets. Dry matter intake of goats fed diet D (7.5% level of AASM), was also significantly higher (P<0.05) than that of control diet (0% AASM) with 61.30g/day. The lowest DM intake was noticed in animals fed diet C containing equal ratio of AASM and GNC (5%). The highest ether extract intake (107.79g/day) recorded for the goats placed on diet B was significantly higher (P< 0.05) than the EE intake of other diets. Ether extract intake of goats fed diet D was also significantly higher (P<0.05) than that of control diet with 4.67g/day, while the goats placed on diet C had the lowest EE intake. Meanwhile, DM intake of all the diets were within the normal range (750 - 1250g/day) recommended for pregnant goats of equivalent body weight (NRC, 1981). With respect to crude protein intake, animals fed control diet had significantly higher value (116.97g/day) than those placed on other diets. Crude protein intake of diet B was however higher than that of diets C and D with 22.11 and 18.77g/day respectively. Crude protein intake in the present study was higher than 9% of the DM intake which was recommended as a minimum CP for pregnant goats (NRC, 2007). This indicated sufficient crude protein for the developing foetus and the dam fed the experimental diets.

The animal fed diet B had 198.70g/day of crude fibre intake which was significantly higher (P< 0.05) than what was observed in other diets. The CF intake of diets D was also higher than that of A and C with 27.92 and 26.04g/day respectively. No significant difference (P>0.05) was observed between the ash intake of the animals placed on diet B and D, while animals fed diet A had a significantly higher (P< 0.05) ash intake (104.42 g/day) than those fed diet C (94.24g/day). Differences observed in the nutrient intake conformed with earlier report (Kronberg and Malechek, 1997) which indicated variation in the ability to adjust to new feed as an important factor influencing intake of feed and nutrient in goats. This may account for the poorer intake recorded for goats fed diet C. Meanwhile, dietary inclusion of ***A. niger*** treated AASM at 2.5 to 7.5 enhanced intake of the pregnant goat in all the nutrients considered aside crude protein. Significantly higher crude protein intake of the control diets (0% AASM) may be a reflection of relatively higher amino acid profile of the groundnut cake (Atteh, 2002) included in the control diet compared. However, higher intake observed in diet B could be an indication of acceptability which explained the probability of higher palatability and or digestibility of the diet (Dumont *et al.,* 1995).

Table 4: Feed intake (g/day) of the pregnant goats fed the experimental diets

	Experimental Diets				
Parameters (g/day)	A	B	C	D	SEM (±)
Dry matter	890.61^{c}	980.23^{a}	789.57^{d}	951.91^{b}	0.009
Crude protein	116.97^{a}	109.39^{b}	87.28^{d}	90.62^{c}	0.036
Crude fibre	169.18^{c}	198.70^{a}	161.06^{d}	197.10^{b}	0.039
Ether extract	102.42^{c}	107.79^{a}	84.90^{d}	107.09^{b}	0.011
Ash	104.42^{b}	112.15^{a}	94.24^{c}	112.58^{a}	0.008

Means on the same row with the same superscript are not significantly different (P>0.05)

Digestibility coefficient and digestible nutrients of the experimental diets are indicated in Table 5. Significant differences (P< 0.05) were noticed across all the parameters studied. Apart from crude protein where diet B had the highest digestibility coefficient (77.48%), the highest value for other nutrients were observed among animals fed diet D. However, the lowest digestibility coefficient and digestible nutrients were recorded for diet C across all the nutrients except crude protein where it superseded only diet D with 1.46% in digestibility coefficient value. The DM and CP digestibility coefficient of diet B was significantly higher (P<0.05) than that of control diet which showed significantly higher (P< 0.05) digestibility coefficient than diet B in CF and EE. Control diet had significantly (P<0.05) higher digestible crude protein (88.05%) than other diets, while digestible dry matter and ether extract were significantly higher (P<0.05) in diets B (807.02%) and D (97.85%) respectively. Diet B and D were however comparable (P>0.05) in their digestible crude fibre which were significantly different (P<0.05) from Control diet and diet C.

Results on digestibility coefficient and digestible nutrients in all the investigated levels of ***A. niger*** treated AASM showed promising nutritive effects on the pregnant goats used for the experiment except diet C. Poorer utilization as recorded for diet C, may likely be associated with poor intake leading to reduced rumination cycle which could consequentially lead to lengthen ingesta passage and poor digestibility (Barboza *et al.,* 2009; Weckerly, 2013). However, better nutrient availability and digestibility observed in diets B and D, particularly on dry matter, crude fibre and ether extract, seemingly indicated safety and beneficial utilization of ***A. niger*** treated AASM which corroborated earlier report by Belewu *et al.* (2015).

Table 5: Apparent digestibility coefficient and mean digestible nutrients of experimental diets

	Experimental Diets				
Digestibility coefficient (%)	A	B	C	D	SEM (±)
Dry matter	81.57^{c}	82.33^{b}	79.92^{d}	83.11^{a}	0.001
Crude protein	75.31^{b}	77.48^{a}	70.99^{c}	69.53^{d}	0.004
Crude fibre	69.94^{d}	75.59^{b}	70.46^{c}	76.33^{a}	0.002
Ether Extract	90.38^{b}	88.76^{c}	88.33^{d}	91.37^{a}	0.003
Digestible Nutrients					
Dry matter	726.47^{c}	807.02^{a}	631.02^{d}	791.13^{b}	0.449
Crude protein	88.08^{a}	84.76^{b}	61.96^{c}	63.01^{c}	0.013
Crude fibre	118.32^{b}	150.20^{a}	113.48^{c}	150.44^{a}	0.025
Ether Extract	92.57^{c}	95.67^{b}	74.99^{c}	97.85^{a}	0.001

Means on the same row with the same superscript are not significantly different (P>0.05)

Conclusion

A. niger treatment of raw Ackee apple seed apparently improved nutrient availability and digestibility of the seed. It is therefore concluded that 7.5% of ***A. niger*** treated AASM can be tolerated by pregnant goat.

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