Dietary Effects of Solid-State Fermented *Jatropha curcas* Kernel Cake on West African Dwart Goats in a Mixed Ration.

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ABSTRACT

In a 120-day trial, sixteen male West African Dwarf (WAD) weaned goats aged 5 to 6 months with an average weight of 6.3± 0.7kg were equally allotted to four experimental diets (A, B, C, D) in a 4 X 4 Latin square design. The four dietary treatments consist of a control, Diet A (100% groundnut cake), Diet B (50% *Absidia spinosa* treated Jatropha *curcas* kernel cake [JKC] + 50% groundnut cake), Diet C (50% *Mucor rouxii* treated JKC + 50% groundnut cake), Diet D (50% treated JKC with the mixed culture of *Absidia spinosa* and *Mucor rouxii* + 50% groundnut cake). The results showed that the dry matter intake for Diets A, B, C and D were 786.37g/d, 277.70g/d, 327.58g/d, and 480.02g/d respectively. The highest dry matter digestibility coefficient was recorded for experimental animals on diet A (70.9%) which was followed by diets D (66.2%), C (65.2%), B (61.9%) in that order. Neutral detergent fibre digestibility for bucks on Diets A, B, C, and D were 78.5%, 57.0%, 64.8%, and 67.9% respectively. Animals on diets A gained 0.08g/day weight while those on diets B, C and D lost weight with mortality of 25.00%, 18.75% and 12.50% respectively. To these effects, a cocktail of fungi improves the nutritive values of feed.

(Keywords: goats, Jatropha, groundnut cake, West African Dwarf goats, digestibility)

INTRODUCTION

Ruminants in the humid tropics of West Africa suffer from seasonal reduction in feed supply and pasture quality. The increasing world population especially in developing countries has resulted in inadequacy of food supply and deficiency of dietary nutrients. The provision of nutritionally balanced ration for both man and livestock is the greatest problem in Nigeria (Bawala and Akinsoyinu, 2002). Adequate nutrition is one of the ways to enhance production of West African dwarf goats.

Livestock are customarily been maintained on feedstuffs that come from three main sources namely: natural rangeland grazing, grown fodder crops and agro-industrial by-products, concentrate supplement is usually not available as part of the nutrition for scavenging animals. It is therefore necessary to explore other sources of carbohydrate, protein and dietary fibre for use in animal nutrition. The need to search for alternative feed components that are feasible, locally available and accessible cannot be over emphasised among tropically cultivated plants. Among these plant species, *Jatropha curcas* seed is considered as a good source of dietary proteins, energy and fibre. In addition to being a source of oil, it also provides a meal that serves as a highly nutritious and economic protein supplement in animal feed (Becker and Makkar, 1998).

The seeds of *Jatropha curcas* are known to be toxic to mice (Adam, 1974) and rats (Stirpe *et al*., 1976). Liberalino *et al*. (1988) found a high degree of toxicity in the raw, cooked or roasted seeds. They found that all rats fed on diets containing different nut fractions died; with raw nuts death occurring within 2-3 days, with raw or cooked nut oil, within 6-8 days; and with roasted nuts, within 14 days. Ahmed and Adam (1979) fed *Jatropha curcas* seeds to six calves at doses of 2.5, 1.0, and 0.25 g/kg once, and to two other calves at 0.025 g/kg up to 14 days. The onset and manifestations of toxicity in the six calves was rapid and death occurred within 19 hours of administration. Though there was an acclaimed success (Becker and Makkar, 1998), the

procedures were complicated and cannot be easily replicated by local farmers. Furthermore, there was the problem of chemical load owing to residues of chemicals used in the extraction of phorbolesters which could have adverse effect on human and animal health.

A crucial obstacle in the establishment of *Jatropha curcas* as a commercial crop could be overcome by detoxifying the kernel cake using other means such as biological method. This method has no adverse effect on both animal and human health. Belewu (2008) showed reduction in some antinutritional factors such as phytate, saponin, and tannin when treated with *Rhizopus oligosporus*. Belewu *et al.* (2010) deduced that *Aspergillus niger* was effective in lowering the level of antinutrients in the kernel cake to a level that does not elicit negative response in the West African Dwarf goats while *Trichoderma longibrachiatum* was not effective in detoxifying the antinutrients as the toxic responses were noticed (persistent diarrhoea, dehydration and sudden deaths). This study seeks to explore other available beneficial fungi that will detoxify *Jatropha curcas* kernel cake. Hence, the thrust of this study was to find a less expensive, accessible and alternative ingredient to groundnut cake by the use of solid-state fermentation in detoxifying *Jatropha curcas* kernel cake.

MATERIALS AND METHODS

Seed Collection

Mature and sun dried seeds of *Jatropha curcas* were collected around Ilorin metropolis, while some were purchased from reputable sources in the same city which is situated in Kwara State, North Central region of Nigeria.

Toasting and Milling

The seeds were decorticated, and the kernels were toasted and milled using the mechanical grinder.

Mechanical Oil Extraction

The Jatropha kernel meal was packed in a sieving cloth which was sown into bags. They were packed on a specially designed wooden oil extractor. The oil was extracted mechanically using hand-driven screw press for about 3 days. A small bowl was placed underneath for easy collection of oil.

Cold Solvent Extraction

The compacted cake was crutched using pestle and mortar. Petroleum spirit was added just above the level of the cake (1:1 v/w) in 5litres white plastics. They were left for 24 hours. The oil layer was decanted, the cake was squeezed in a sieving cloth and sun dried.

Solid State Fermentation

30mls of distilled water was added to 20g of Jatropha kernel cake (3:2 v/w) in a covered bowl, which was earlier disinfected using ethanol. It was kept in a dark room for 5 days at room temperature to allow natural solid-state fermentation to occur. Different colonies of fungi were observed to have grown on the substrate.

Media and Culture Conditions

The pin mouth was used to pick from different colonies and spread on the sarbroud agar in 5 bottles. They were left for 5 days to allow proper growth of the micro-organisms. Stock cultures of fungi were later maintained at $4^{o}C$ on commercially prepared Potato Dextrose Agar.

Identification and Isolation of Microorganisms

Lactophenol blue was used to stain a slide. Pin mouth was used to pick from each colony and placed on the slide. Then, slide cover was placed on each slide. This was observed under the microscope (Olympus CX21) at a magnification of X40. After several observations, *Rhizopus sp., Absidia spinosa., Aspergillus sp., Mucor rouxii* and *Klebsilla oxytoca* were identified.

Pure Culture

Mucor rouxii and Absidia spinosa were tissue cultured to obtain homogenous samples of fungal mycelia. The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

Sub-Culturing Method

20g of potato dextrose agar was weighed into two 250mls conical flasks. 250mls of distilled water and 0.3 mls on streptomycin were added to each flask. They were immediately covered with cotton wool and aluminium foil and autoclaved at 121^{0}C for about 15 minutes. They were placed in cold water. About 10mls of the prepared agar medium was poured into each 40 petri dishes and were allowed to solidify. Inoculating pin was used to place part of the colony on the medium. It was flamed when switching over to the second fungus. The inoculated media were kept in the dark cupboard in the laboratory for 5 days at 30^{o}C and 100% RH (Relative Humidity).

Inoculation and Incubation of Substrate

Spores from 5 days old agar slants were collected by adding sterile distilled water. Three big black bowls were disinfected with ethanol after which the autoclaved Jatropha curcas kernel cake was inoculated separately in layers with the fungi (*Absidia spinosa* and *Mucor rouxii*) using the dilution and later incubated at room temperature.

Absidia spinosa and *Mucor rouxii* were harvested from thirteen petri dishes each to inoculate two bowls while 7 plates of each fungi culture (plate size 10cm diameter) were mixed in the suspension to inoculate the third bowl. They were moistened with distilled water to aid proper growth of the fungi. On the seventh day, the fungi were observed to have enveloped the substrate. White and dark-grey growth was observed to have covered the substrate inoculated with *Mucor rouxii* while greyish growth was noted on the substrate inoculated with *Absidia spinosa*. They were mixed properly and left for another 7 days. The growth was terminated by autoclaving in a foil paper.

Diet Formulation

The spent substrate was used in the formulation of diets for the experimental goats at inclusion levels of 0% and 50% in replacement for groundnut cake in a mixed ration. Three other ingredients were kept in same proportion.

Experimental Animals and Management

Sixteen (16) yearling West African Dwarf (WAD) bucks of an average weight of 6.3±0.7kg, were purchased from reputable sources. Prior to the start of the experiment, they were administered with a broad spectrum antibiotic (Oxytrox 20% L.A injection, dose: 1ml per 10 kg) and dewormed using Albidol orally. Promectin was also given at a dose of 1ml per 10 kg to eliminate possible endo- and ecto- parasites by a vetenarian. Pens were washed and disinfected.

Table 1: Experimental Diets Fed To West African Dwarf Goats

Ingredients (%)	DIET A	DIET B	DIET C	DIET D
Cassava peels	57.00	57.00	57.00	57.00
Rice Bran	19.00	19.00	19.00	19.00
Wheat Offals	14.00	14.00	14.00	14.00
Groundnut cake	8.00	4.00	4.00	4.00
Fungus treated JKC	0.00	4.00	4.00	4.00
Salt	1.00	1.00	1.00	1.00
Vitamin-mineral premix	1.00	1.00	1.00	1.00
Total	**100**	**100**	**100**	**100**

JKC = Jatropha Kernel Cake
Diet A = Control diet
Diet B = Jatropha kernel cake treated with *Absidia spinosa*
Diet C = Jatropha kernel cake treated with *Mucor rouxii*
Diet D = Jatropha kernel cake treated with *Absidia spinosa* and *Mucor rouxii*

The bucks were randomly divided into four equal groups in a 4 X 4 Latin square design model, and allotted to four different diets A, B, C, and D with adjustment period of 10 days. The feed offered and orts were recorded daily and animals were watered *ad libitum* for 120 days. They were weighed weekly to determine their growth rate. Digestibility coefficient study was carried out in the last week of the experiment using total fecal collection method (Belewu and Adenuga, 2003).

Chemical Analysis

The proximate composition of the four diets (A, B, C, and D) and the fecal samples were determined according to the method described by AOAC, (1995) while the fiber fractions were determined according to the method of Goering and Van Soest, (1970).

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) of a 4 x4 Latin square design model and mean separation was by Duncan multiple range tests (1955) using Statistical Analysis System (SAS) 1999 package.

RESULTS AND DISCUSSION

The crude protein (CP) content of 9% to 9.6% in the diets was within the normal requirement of weaned goats. Apori (1988) suggested a range of 7% to 12% C P which he believed to be adequate. NRC (1985) reported a minimum of between 6 and 8% CP for maintenance purpose in ruminant animals. The mode of feed presentation and crude protein content can stimulate the appetite and feed intake thus the high value of intake for the control diet. This can be deduced that more of a diet with high crude protein would be consumed compared with low crude protein content (Taiwo and Anosa, 1995).

Table 2: Proximate Composition of Experimental Diets on Dry Matter Basis.

Parameters	DIET A	DIET B	DIET C	DIET D	±SEM	P-VALUE
Dry Matter	95.18	94.44	96.55	94.43	1.00 ns	0.925
Crude Protein	9.00	9.60	9.20	9.60	0.44 ns	0.971
Ether Extract	9.00	8.30	7.90	8.60	0.48 ns	0.941
Ash	8.50	8.70	8.63	9.44	0.49 ns	0.945
Crude Fibre	20.04	21.02	19.36	22.59	1.37 ns	0.913
Nitrogen Free Extract	48.64	46.82	51.46	44.20	1.67 ns	0.544
Neutral Detergent Fibre	53.76	50.05	52.02	51.61	1.38 ns	0.894
Acid Detergent Fibre	18.24	15.48	12.54	15.00	1.49 ns	0.718
Hemicellulose	35.52	34.57	39.48	36.61	1.46 ns	0.759
Lignin	2.05	2.72	3.30	3.00	0.35 ns	0.736
Cellulose	13.11^{c}	9.64^{b}	6.27^{a}	9.05^{ab}	0.97	0.020
Silica	3.08	3.12	2.97	2.93	0.31 ns	0.998

a,b,c, Means within a row with different superscripts are significantly different ($p < 0.05$) ns = not significantly different ($p>0.05$).

Table 3: Nutrient Intake of the Fungi Treated *Jatropha Curcas* Kernel Cake Based Diets by WAD Goats.

Parameters	DIET A	DIET B	DIET C	DIET D	±SEM	P-VALUE
Dry Matter Intake (g/d)	786.37^{d}	277.70^{a}	327.58^{b}	480.02^{c}	75.35	0.000
Crude Protein Intake (g/d)	74.36^{c}	28.23^{a}	31.21^{a}	48.80^{b}	7.00	0.004
Ether Extract Intake (g/d)	74.36^{c}	24.41^{a}	26.80^{a}	43.72^{b}	7.68	0.003
Ash Intake (g/d)	70.23^{c}	25.58^{a}	29.28^{a}	47.99^{b}	6.87	0.004
Crude Fibre Intake (g/d)	165.57^{a}	61.81^{c}	65.69^{c}	114.83^{b}	15.97	0.000
Nitrogen Free Extract Intake (g/d)	401.86^{d}	137.56^{a}	174.60^{b}	224.68^{c}	38.33	0.000
Neutral Detergent Fibre Intake (g/d)	444.16^{d}	147.17^{a}	176.50^{b}	262.35^{c}	43.67	0.000
Acid Detergent Fibre Intake (g/d)	150.70^{c}	45.52^{a}	42.55^{a}	76.25^{b}	16.54	0.000
Hemicellulose Intake (g/d)	293.46^{d}	101.65^{a}	133.95^{b}	186.10^{c}	27.54	0.000
Lignin Intake (g/d)	16.94	8.00	11.20	15.25	2.01^{ns}	0.478
Cellulose Intake (g/d)	108.31^{c}	28.35^{a}	21.27^{a}	46.00^{b}	13.06	0.000
Silica Intake (g/d)	25.45	9.17	10.08	14.89	2.88^{ns}	0.133

a,b,c, Means within a row with different superscripts are significantly different ($p < 0.05$) ns = not significantly different ($p>0.05$).

The goats fed various treatment diets showed positive DM status by consuming at least 1.5-3% of their body weights which is the recommended daily dry matter requirement for meat type goats in the tropics (Devendra and Mcleroy, 1982). The dry matter intake of fungi treated Jatropha kernel based diets observed was close to that reported by Belewu *et al.*(2010). Variations within breed may occur due to nutrition, environmental conditions and season of study.

The highest DMI was from Diet A, probably because the combination was palatable. This suggests that goats would benefit more from being fed diet A in approximately this proportion. This makes biological sense in terms of nutrient density of the diet because it suggests that an animal would tend to consume more of the diet in order to derive more of the needed N for biological activities. This is supported by Rajpoot *et al.* (1981), Malachek and Provenza (1981) who had earlier reported that the low N content of feeds significantly reduced the DMI from such feeds. The results of the present study, however, suggest that the relationship between dietary N content of feed and the feed DMI was strong.

Earlier observation showed that intake in ruminants is also influenced by a taste related factor-palatability. Beyond nutritional composition, animals tend to consume more of palatable diet (Preston and Leng, 1986). Sensory factors such as the perceived odour could affect feed intake when other factors are recessive. An inverse relationship has long been reported between the DMI and the fiber content of feed (Reid and Klopfenstein, 1983).

Goats on Treatment D had DM intake of 480.02g/d. which shows that the mixture of the *Absidia spinosa* and *Mucor rouxii* tend to improve the odour and taste of the diet. The combination of these fungi renders diet D the best option for maximum DMI by goats among others in treating the Jatropha kernel cake. However, it is presumed that nutrient density may be the over-riding factor influencing DMI in this study. This may very well explain the rise in DMI just as the treated Jatropha kernel cake contents of the diets improved from C, B, and D

Table 4: Digestibility Coefficients of WAD Goats Fed Fungi Treated *Jatropha Curcas* Kernel Cake Based Diets.

Parameters	DIET A	DIET B	DIET C	DIET D	±SEM
Dry Matter Digestibility %	70.90	61.90	65.20	66.20	1.70 ns
Crude Protein Digestibility %	58.10^{b}	30.00^{a}	37.20^{a}	40.90^{a}	4.07
Ether Extract Digestibility %	64.10^{b}	44.90^{a}	44.00^{a}	60.30^{b}	3.61
Crude Fibre Digestibility %	83.90^{b}	68.70^{a}	74.10^{ab}	80.60^{ab}	2.48
Nitrogen Free Extract Digestibility %	72.30	77.90	78.20	73.60	1.55 ns
Neutral Detergent Fibre digestibility %	78.50^{b}	57.00^{a}	64.80^{a}	67.90^{ab}	3.13
Acid Detergent Fibre Digestibility %	62.00	51.60	56.60	59.30	1.85 ns
Hemicellulose Digestibility %	87.00^{b}	59.40^{a}	67.30^{a}	71.40^{a}	3.96
Lignin Digestibility %	75.50	79.10	81.70	80.90	1.47 ns
Cellulose Digestibility %	51.00^{c}	28.10^{ab}	22.90^{a}	38.90^{b}	4.23
Silica Digestibility %	61.00^{b}	57.60^{b}	31.90^{a}	65.80^{b}	5.10

a,b,c, Means within a row with different superscripts are significantly different ($p < 0.05$) ns = not significantly different ($p>0.05$).

The digestibility values obtained for the nutrients in all the diets suggest that the fungi treated Jatropha kernel cake based diets and the control were degraded in the rumen. There was significant difference in the dry matter and crude protein digestibility among the diets, although the diets had different nutrient concentrations. Nutrient digestibility was high with the inclusion of a mixture of *Absidia spinosa* and *Mucor rouxii* treated Jatropha kernel cake based diet when compared with diets B and C.

Crude protein, ether extract and crude fibre are components of DM and therefore any factor that affects the DM of a feed would similarly affect the CP and CF component of same feed. This may explain why the digestibility coefficients of CP, EE and CF increased in diets A and D.

The highest digestibility coefficient of NDF and ADF was by goats on Treatment A. Cassava peel, wheat offals and rice bran provided most of these to the experimental animals. The coefficient of digestibility for ADF and lignin were significantly lower in the fungus treated Jatropha kernel cake based diets B and C. This was probably because the 50% supplementation resulted in gastrointestinal disorder which the treated cake alone is prone to induce.

The fecal CP values observed among treatment groups is in consonance with the findings of Black *et al*., (1978) who observed that fecal nitrogen was not significantly affected by nitrogen intake. The significant fecal nitrogen values observed among treatment groups may be attributed to variation in nitrogen metabolism.

The deamination process in the rumen may have produced more ammonia from the diets relative to the control, probably due to the nature and quality of their dietary protein. According to Ranjah (1980), the concentration of ammonia in the rumen fluid would depend on the quantity and solubility of protein fed to the animals. Nitrogen excreted in feces therefore would depend on urea recycling effect, the efficiency of utilization of ammonia produced in the rumen for microbial protein synthesis, microbial load balance and the amount of by-pass protein that tannin can give rise to. It is possible that the protein moiety of the diets B, C, and D was more soluble than that of the control. That also meant that more rumen ammonia would be produced which surely would have increased as the nitrogen intake increased from diets D to B.

The weight gain observed in diet A might be due to the higher intake of nitrogen and energy. The improvement in the body weight gain could be explained by the fact that feed intake by goats, in relation to its weight gain, tends to increase as it grows rapidly with improved feed efficiency. The average daily weight gain may show changes in rumen fill as much as changes in body tissue (Aregheore, 2007). Weight loss as recorded in this study could be attributed to the level of other antinutrional factors which were not detoxified by the fungi such as phorbolesters (Begg and Gaskin, 1994).

Table 5: Organs' Evaluation of WAD Goats Fed *Jatropha Curcas* Kernel Base Diets.

Parameters	DIET A	DIET B	DIET C	DIET D	±SEM
Live weight during slaughtering (g)	7000.00	5000.00	4700.00	4900.00	4.67 ns
Avg. daily feed intake (g/d)	826.19^{d}	294.05^{a}	339.29^{b}	508.33^{c}	78.98
Daily weight gain/loss (g/d)	0.08	-0.14	-0.13	-0.09	42.06 ns
Heart (g)	647.40^{d}	192.00^{a}	235.50^{b}	296.00^{c}	67.97
Spleen (g)	60.60^{b}	59.60^{b}	37.90^{a}	36.70^{a}	4.52
Liver (g)	131.60^{a}	450.30^{d}	305.50^{c}	287.70^{b}	42.68
Kidney (g)	126.00^{c}	102.00^{b}	69.90^{a}	91.90^{b}	7.72
Lung (g)	557.60^{d}	523.00^{c}	305.40^{a}	320.50^{b}	43.29
Large intestine (g)	4500.10^{d}	1658.60^{b}	1187.00^{a}	3189.40^{c}	4.94
Empty Large Intestine (g)	1000.00^{d}	704.00^{b}	433.60^{a}	784.30^{c}	76.63
Small Intestine (g)	2139.70^{d}	1890.80^{c}	1012.90^{a}	1694.00^{b}	1.58
Empty Small intestine (g)	1553.00^{d}	1034.20^{c}	728.10^{a}	933.60^{b}	1.14
Mortality(%)	0.00	25.00	18.75	12.50	3.49 ns

a,b,c, Means within a row with different superscripts are significantly different ($p < 0.05$) ns = not significantly different ($p>0.05$).

Goats fed with Treatments B, C and D were weak and emaciated. This is in agreement with Chivandi *et al.* (2006) who noticed a similar trend in pigs; Belewu (2008) in albino rats; El-Badwi *et al.*(1995) in Brown Hissex chicks and humans (Makkar *et al*., 1997). Diarrhoea, reduced feed and water intake, dehydration and death were noticed first in the animals on Treatments B later C and D on the 3rd, 6th, and 11th week of the study respectively. This agrees with the reports of Aregheore *et al.* (2005) on sheep and goats, El-Badwi *et al.* (1995) on chicks.

CONCLUSION

Treating Jatropha kernel cake with a cocktail of *Absidia spinosa* and *Mucor rouxii* resulted in improved Dry Matter, Crude Protein, Acid Detergent Fibre and Lignin intake and nutrient digestibility. The use of *Absidia spinosa* and *Mucor rouxii* singly in inoculation of *Jatropha curcas* kernel cake was not adequate in detoxification as it elicited negative responses. Generally, nutrient utilization, immunity, and performance were not improved.

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