Trop. J. Anim. Sci. 2003 Vol. 6 No. 1: 137-143

# Effects of Detoxification on Nigerian Neem Kernel Composition and Its Impact on Swine Performance

**A.A. Annongu [1,2*] U. ter Meuleu[2], F. Liebert[2], J.O. Atteh[1] and J.K. Joseph[1].**

*[1]Institute for Animal Physiology and Animal Nutrition, University of Gottingen, 37077 Gottingen, Germany; [2] Laboratories Of Nutritionbiochemistry & Physiology, Dept of Animal & Poultry, PMB 1515, University of Ilorin, Ilorin, Nigeria, West Africa*

**Target Audience:** Nutritionists, feed toxicologists, farmers and animal scientists.

## Abstract

*Whole neem Whole neem kernels were milled into flour and subjected to natural lactic fermentation and lyle (unconventional alkali) treatments. Twelve weanling Large white x Duroc piglets with an average weight of 12.5 ± 0.5 kg weight were housed in individual stalls. Four diets were formulated for the experiment. A corn-soy reference diet and three diets containing 20 and 30% raw neem kernel meal (NKM) and 30% treated NKM. Each treatment consisted of three stalls, each with one piglet. Pigs were fed to appetite for twenty one days. The raw NKM contained high concentration of phytotoxins, low protein-nitrogen and lacked some essential amino acids, namely lysine and threonine. Treating the NKM reduced these defects. The performance of the pigs as measured by feed consumption, growth rate or feed efficiency gave some improvement on diet with treated NKM ($p > 0.05$). Untreated NKM diets produced poor results on all the performance criteria ($p < 0.05$) relative to the treated NKM or control diet. It appeared that the treatment could not eliminate the bitter taste associated with neem alkaloids as the pigs consumed less than those on the standard diet. To make the NKM a suitable feedstuff for swine, treated neem meal might be included in diet mixture at lower levels from 10 to 20% inclusion.*

**Key words:** Neem kernel meal, swine, treatments performance

## Description of Problem

Research on the use of alternative unconventional feedstuffs by livestock will continue to receive attention because of the persistent world food problems. The search for the alternatives calls for the sourcing of novel feed stuffs or by-products from agricultural or industrial origin. The relative abundance of the neem tree (*Azadirachta indica* A. Juss) and the possibility of using its products formed the basis for the present study, Previous works (1, 2, 3, 4) have revealed that neem tree and its products contain secondary metabolites collectively called azadirachtins, including nimbin, nimbidin, nimbinin and nimbosterol. However, the azadirachtins, like other neem compounds, include many analogues (5). Besides these, the neem tree also harbours alkaloids, salannins, desacetylsalannin, desacetylnimbin (6), six heat-stable phenolic compounds, seven epoxy compounds, flavanols, sterols and ketones (7). Of all the chemical constituents of the neem tree, the azadirachtins are the most biologically active, followed by the salannins (8).

* Author for correspondence

Extracts of neem seeds at concentration as high as 8500mg/kg body weight proved non-toxic to rats (9, 10) and no toxicity was observed in albino rats and mice fed nimbin at 2000mg/kg orally or 100mg/kg intra-peritoneal. Human subjects receiving neem seed oil against certain infections experienced occasional nausea and general discomfort (11). Studies (12) have shown that pigs fed on a diet containing 10% water-washed neem seed cake in place of groundnut cake utilised the feed efficiently and gave a performance comparable to the control diet. In this study, whole neem kernel, grown in Nigeria and believed to vary in activity was treated by chemical and biochemical means, followed by a comparative investigation of its feeding potential in the nutrition of swine.

## Materials and Method

*Collection and processing of neem kernels*

Neem kernels were obtained by shaking them off or stripping them from the branches or by picking up from the ground clean ripe fruits that had fallen naturally to the ground at maturity, After collection, the pulps were removed and rinsed then the kernels sun-dried, after which they were milled into flour. The meal was subjected to natural lactic fermentation and treatment with *lyle* (unconventional alkali) following the outlined procedures (13). Preparation of the lyle was carried out using parkia pod husks (from *Parkia filicoides*) which have an exceptional purity of alkalinity. The husks were burnt to ashes and several kilograms of the ash was mixed with tap water and allowed to extract for a week. The resulting *lyle* was filtered using a muslin, producing an unconventional alkali solution (mainly potash) with a pH of 11.5. NKM was soaked in the *lyle* for 48h after which the dough was removed, strained and sun-dried to constant weight prior to incorporation in diets.

*Experimental procedures*

Twelve Large white x Duroc weanling pigs of 12.5 0.5 kg live weight were housed in individual stalls. Four iso-energy and iso-nitrogenous diets were formulated comprising a corn-soy reference diet and three diets containing 20, or 30% raw neem kernel meal, or 30% treated neem kernel meal (Table 1). The experiment was designed as a one-way classification. There were four treatments and for each of the four treatments, were three replicate stalls each with a pig. The pigs were randomly allotted to the treatments and fed to appetite the diets shown in Table 1.

**Table 1. Composition of the experimental diets (%)**

| Dietary treatments | 1 (0% NKM) | 2 (20% NKM) Untreated) | 3 (30% NKM) untreated) | 4(30% NKM) treated) |
|---|---|---|---|---|
| **Ingredients** | | | | |
| Maize | 78.58 | 71.40 | 67.56 | 67.56 |
| Soyabean meal | 18.98 | 6.16 | - | - |
| Neem kernel meal | - | 20.00 | 30.33 | 30.00 |
| Dicalcium phosphate | 0.84 | 0.84 | 0.84 | 0.84 |
| Ground limestone | 0.80 | 0.80 | 0.80 | 0.80 |
| Sodium chloride | 0.40 | 0.40 | 0.40 | 0.40 |
| *Premix | 0.40 | 0.40 | 0.40 | 0.40 |
| Total | 100 | 100 | 100 | 100 |

*Mineral-vitamin premix: supplied per 2.5kg/tonne of feed, 10,000000IU vitamin A; 3000000IU vitamin D3; 8000IU vitamin E; 2000mg vitamin K; 2000mg vitamin B1; 5500mg vitamin B2; 12mg vitamin B12; 10,000mg niacin; 100m selenium; 1200mg vitamin B6; 10,000mg vitamin C; 6000mg antioxidant; 7000mg pantothenic acid; 600mg folic acid; 500,000mg choline chloride; 60,000mg iron; 80,000mg manganese; 8000mg copper; 50,000mg zinc; 450mg cobalt; 2000mg iodine; 30mg biotin and 100,000mg magnesium.

The feeding trial lasted three weeks. The experimental pigs were weighed at the commencement of the experiment and thereafter every week. Feed consumption, growth rate, feed efficiency and mortality rate were recorded during the trial.

*Chemical analyses*

Analysis of the nutrient content of diets, the proximate composition of raw and treated neem kernel meals were carried out as described (14). Quantitative determination of azadirachtin A, desacetylsalannin, nimbin and salannin was conducted using high pressure liquid chromatography, whereby the quantities of the toxicants were achieved through the use of external standards and valley to valley integration (Trifolio-M, 2000). The protein-nitrogen in both the raw and processed neem kernel meals was determined using the automated FP-2000 protein/nitrogen analyzer (Weende experimental station, Goettingen, Germany). Amino acid profile of both treated and untreated neem kernel meal was carried out employing the Eppendorf (Biotronik) LC 3000 amino acid analyser. Replicated samples were hydrolysed with hydrochloric acid (Hcl) followed by determination of amino acid on cation exchange system. Similar samples were oxidized with performic acid followed by hydrolysis with Hcl and amino acid determined. Two methods of analysis were used to ensure that some amino acids destroyed in one method could be obtained in the other. The average of the four determinations gave the total amino acid content of the treated and raw neem kernel meal in g/16gN.

*Statistics*

All data collected were subjected to one-way ANOVA and means were tested for significance at 5% confidence level using the Duncan's Multiple Range Test (15).

## Results and Discussion

Table 2. Chemical constituents of raw and treated neem kernel (NKM) meal determined by HPLC* (mg/g)

| Chemical constituents | Raw NKM | Treated NKM |
|---|---|---|
| Azadirachtin A | 2.380[a] | 0.141[b] |
| Desacetylsalannin | 0.812[a] | 0.040[b] |
| Nimbin | 1.077[a] | 0.049[b] |
| Salannin | 3.484[a] | 0.125[b] |

a, b: Treatment means in rows followed by different letters are significantly different ($p<0.05$)

*HPLC: High pressure chromatography, reversed-phase procedure.

Table 2 shows a summary of the results of the chemical constituents in raw and treated NKM. Untreated neem had high concentrations of azadirachtins, desacetylsalannin, nimbins and salannins. Treatments reduced the amounts of these chemical compounds to negligible levels. The quantitative assays of the chemical constituents thus showed that the concentrations of azadirachtins and the other chemical compounds with their analogues in the raw Nigerian NKM were very high. These results are in line with previous reports (16, 17) in which the azadirachtins contents of 19 and 66 neem kernel samples from two different geographical locations were compared and their dependence on temperature, relative humidity and light was observed. These authors also found high contents of azadirachtins in samples from Togo. Togo and Nigeria are located in the same geographical region of Africa. After subjecting raw NKM to lactic fermentation coupled with lyle treatment, the chemical compounds in neem were significantly reduced. Adequate treatment of neem and its products is thus necessary if its utilization as an alternative feedstuff is desired. The use of a cocktail of methods in processing neem products has been demonstrated to effect detoxification (18, 19). Other methods of treatment incidentally employed in this study include ultraviolet photolysis when the meal was sun-dried. Photo-degradation of azadirachtins has been reported under field condition (20, 21, 22).

Another possible reason why the treated NKM had its content of chemical compounds reduced is that phytotoxins like azadirachtins have been shown to be broken down by enzymes (23, 24) and by microbial hydrolysis (25, 26).

Table 3. Proximate composition and gross energy of raw and treated neem kernel meal (NKM) in %

**Table 3. Proximate composition and gross energy of raw and treated neem kernel meal (NKM) in %**

| Sample | Dry matter | Crude protein | Ether extract | Crude fibre | Total ash | Gross energy (KJ/kg) |
|---|---|---|---|---|---|---|
| Raw NKM | 89.76 | 18.94 | 20.68 | 23.01 | 2.02 | 23070 |
| Treated NKM | 87.45 | 19.75 | 18.05 | 22.00 | 2.65 | 21858 |
| | NS | NS | NS | NS | NS | NS |

NS, No significant difference (P>0.05)

The anaerobic fermentation and lyle treatments increased the nutrients content of the treated neem kernel meal (Table 3) with respect to protein and minerals (ash). The treatment also caused some significant degradation of the crude fibre in the raw NKM.

**Table 4. Percentage nitrogen and amino acid composition in raw & treated neem kernel meal (DM)**

| Parameter | Raw | Treated |
|---|---|---|
| Nitrogen average | 3.03 | 3.15 |
| Nitrogen s. deviation | 0.12 | 0.11 |
| Nitrogen c. of variation | 4.03 | 2.05 |
| Number of points | 3 | 3 |

**Table 5**

| Amino acid | | | | | |
|---|---|---|---|---|---|
| **Essential** | mg/g | g/16gN | Mg/g | g/16gN | |
| Isoleucine | 4.33 | 3.38 | 5.50 | 3.55 | 4.00 |
| Leucine | 8.31 | 6.49 | 10.62 | 6.86 | 7.00 |
| Lysine | 4.79 | 3.10 | 5.26 | 3.40 | 5.50 |
| *Methionine | 2.04 | 1.32 | 2.08 | 1.34 | |
| *Cystine | 3.34 | 2.16 | 3.54 | 2.69 | |
| *Phenylalanine | 4.43 | 3.46 | 6.14 | 3.97 | |
| *Tyrosine | 2.47 | 1.93 | 3.27 | 2.11 | |
| Threonine | 3.77 | 2.94 | 5.01 | 3.24 | 4.00 |
| Tryptophan | 1.45 | 0.91 | 1.55 | 0.94 | 1.00 |
| Valine | 6.36 | 4.97 | 8.36 | 5.40 | 5.00 |
| **Non-essential** | | | | | |
| Aspartic acid | 10.37 | 8.11 | 14.83 | 9.58 | |
| Glutamic acid | 29.36 | 22.94 | 40.10 | 25.90 | |
| Alanine | 5.06 | 3.95 | 6.77 | 4.35 | |
| Arginine | 7.38 | 5.77 | 13.64 | 8.81 | |
| Glycine | 5.17 | 4.04 | 6.31 | 4.08 | |
| Histidine | 1.90 | 1.47 | 3.16 | 2.04 | |
| Proline | 5.11 | 4.00 | 6.69 | 4.32 | |
| Serine | 5.36 | 4.19 | 5.37 | 4.22 | |

*Methionine+cystine, FAO/WHO requirement is 3.50g/16g N;
Phenylalanine+tyrosine, the requirement is 6.00g/16g N

Protein-nitrogen in the meal was increased by the fermentation treatment (Table 4) from 3.03 in raw meal to 3.15 in treated. Table 5 presents data on amino acid profile of raw and treated NKM compared to a reference pattern (27). Neem kernel meal was deficient in some essential amino acids such as lysine and threonine. Treatment by fermentation increased the protein content (from 18.94 in raw meal to 19.75 in treated meal) with a subsequent increase in the concentration of some amino acids. The findings that treatments reduced the high fibre content in NKM, increased protein-nitrogen and the level of some essential and non-essential amino acids that resulted from the increase in protein agreed with the works of past authors (28, 29, 30) who showed that fermentation increases protein and vitamin contents and also brings about detoxification as well as improving palatability and odour.

**Table 6. Influence of dietary treated & untreated neem kernel meal on performance of swine**

| Dietary treatment | 1 | 2 | 3 | 4 | SEM | NRC (1998) |
|---|---|---|---|---|---|---|
| **Performance** | | | | | | 10-20kg *bwr |
| Feed intake (g/pig/day) | 931.40[a] | 553.93[c] | 505.80[c] | 700.40[b] | 17.34 | 1000 |
| Weight gain (g/pig/day) | 406.10[a] | -33.20[d] | -152.40[c] | 254.04[b] | 1.88 | 475 |
| Feed efficiency (Gain: Feed) | 0.45[a] | -0.1[c] | -0.34[b] | 0.36[a] | 0.027 | 0.498 |
| Mortality | 0 | 0 | 0 | 0 | 0 | 0 |

a, b, d: Treatment means in rows followed by different letters are significantly different (p<0.05), *bwr, body weight range.

The performance of the weanling pigs fed treated or untreated NKM diets compared with that on the control diet is shown in Table 5. Pigs consumed more of the diet containing processed neem meal than of those with untreated meal (P<0.05). However, there was a significant difference in feed intake between the pigs receiving diet with treated neem meal and those given the standard diet (P<0.05). Body weight gain was highest on the control diet followed by the treated NKM diet (P<0.05). Pigs maintained on the raw NKM diets lost weight and increasing the level of raw NKM in diets increased weight loss (P<0.05). Feed conversion ration followed a treand similar to weight gain. The efficiency of feed utilization of pigs on the treated neem meal was not significantly different from that on the standard diet (P<0.05). No mortality was recorded on all the treatments but pigs offered untreated neem meal based diets emaciated. The experimental pigs consumed more of the feed containing treated NKM than those pigs fed diets with raw NKM because neem kernels contain bitter alkaloids with unpleasant odour (31). These negative attributes of raw neem products may be responsible for the depression in feed consumption observe in the present study. It has been shown that some seeds are not usually used as food in their unfermented state because of their toxic or anti-nutritional factors content (32). However, when treated, they become edible and can contribute to nutrient intake, hence the significant increase in feed consumption recorded in the present work on pigs fed treated neem kernel meal. However, while pigs on the control diet gained 450 g/d, those on the treated neem meal based diet gained only about 250g. This was probably due to the presence of the antinutritional factors present in neem based diets which might have prevented the efficient feed utilization and body weight gain. The loss of weight by pigs fed raw NKM agreed with the report of past investigators (33) who found that lambs given raw neem cake feed lost weight. Observations on this study are also consistent with previous findings (34) that feeding neem seed based diets to cattle resulted to depression in feed efficiency and growth. No mortality was recorded on any of the treatments but pigs maintained on raw KNM diets became severely emaciated which forced the experiment to be terminated before the time scheduled for completion.

Further research into digestibility of raw and treated NKM by swine is recommended including the possibility of treating neem products with polar and non-polar solvents to remove the bitter taste and supplementation with amino acids.

## Acknowledgement

This research work was supported with grant from Katholischer Akademischer Auslaender-Dienst (KAAD), Germany, for which the authors are earnestly grateful.

## References

1. Gimlette, J.D. 1929. Malar poisons and charm cures, 3rd Ed. 301 pp London.
2. Siddiqui, S. 1942. A note on the isolation of three new bitter principles from the nim oil (*Azadirachta indica*). Curr. Sci. (Bangore) 11: 278-279.
3. Bhattacharji, S.C., Mitra, Siddique, S. 1949. A note on the chemical extraction of nim (*Melia azadirachta*) trunk-bark. Chem. Abs. 44: 3097.
4. Sengupta, P.S., Choudhuri and Khastgir. 1958. Investigation of the trunk-bark of M. azadirachta. Chem. Abstr. 53: 1400.
5. Rembold, H., Forster, H. and Czoppelt, Ch., 1986. Structure and biological activity of azadirachtins A & B. Proc. 3rd Int. Neem Conf., Nairobi pp. 149-160.
6. Tirimanna, A. S. L. 1984. Surveying the chemical constituents of the neem leaf by two-dimensional thin-layer chromatography. Proc. 2nd. Int. Neem Conf., Rauishholzhause, Germany, pp. 67-74.
7. Kraus, W., Bauman, S., Bokel, M., Keller, U., Klenk, A., Kligele, M., H. and Schwinger, M. 1986. Control of insect feeding and development by constituents of melia azedarach and *Azadirachta indica*. Proc. 3rd Int. Neem Conf., Nairobi, pp. 111-125.
8. Ascher, K.R.S. and Gsell, R. 1981. The effect of neem seed kernel extract on *Epilachna varivestis* Muls. Larva. Z. Pflkrankh. Pflschutz. 88: 764-767.
9. Feuerhake, K. and Schmutterer, H. 1982. Einfache Verfahren zur Gewinnung und Formulierung von Neemsammenextrakten und deren Wirkung auf verschiedene Schadinsekten. Z. PflSchutz. 89: 737-747.
10. Lange, W. and Feuerhake, K. 1984. Wirkungssterigerung von angereicherten Neemsamenextraken durch den synergisten Piperonylbutoxid unter Labordedingungen. Z. PflKrankh. Pflschutz. 91.
11. Chopra, I.C. 1965. Poisonous plants of India, vol. 1. Indian Agric. Res. Inst. New Delhi p.245.
12. Sastry, V.R.B. and Agrawal, D.K. 1992. Utilisation of water-washed neem (A. indica) seed kernel cake as a protein source for growing pigs. J. Appl. Anim. Res. 1: 103-107.
13. Annongu, A. A., ter Meulen, U., Atteh, J. O. and Apata, D. F. 1996. Toxikologische Beurteilung von nativem und industriell fermentiertem Schibutter-Olkuchen fur die Futterung von Broilern. Arch. Geflugelk 60(5): 221-226.
14. AOAC. 1990. Assoc. Off. Anal. Chem.: Helrich, K. (Ed), 15th Ed., Arlington, Vol. II 800.
15. Duncan, D.B. 1955. Multiple range & multiple F. tests. Biometrics, 11: 1-42.
16. Schmutterrer, H. and Zebitz, C.P.W. 1984. Effect of methanolic extracts from seeds of single neem trees of African and Asian origi on E. varivestia and Aedes aegypti. Proc. 2nd. Int. Neem Conf., Rauishholzhausen, Germany, pp. 83-90.
17. Ermel, K., Pahlich, E. and Schmutterer, H. 1986. Azadirachtin content of neem kernels from different geographical locations, and its dependence on temperature, relative humidity and light. Proc. 3rd int. Neem Conf., Nairobi, pp. 33-44.
18. Delaude, C. 1974. Chemical study of the saponins of Sapindacaea. Identification of the sapoinn of *Radikofera calodendra*. Bull. Soc. R.Sci. Liege 43: 693-696.
19. Mandrile, E.L. and Digiusto, M. I. 1977. Saponosides in *Polygala stenophylla*. A. Gray Rev. farm (Buenos Aires) 119: 7-9.

20. Kitagawa, I., Yoshikawa, M., Imamura, Y. and Yoshioka, I. 1974. Saponins and sapogenols. VIII. Photochemical cleavage of glycoside linkage in saponin 1. Photolysis of some saponins and their structural features. Chem. Pharm. Bull. 22: 1339-1347.

21. Ladd, T. L. Jr., Jacobsom, M. and Buriff, C.R. 1978. Japanese beetle: extracts from neem tree seeds as feeding deterrent. J. Econ. Ent. 71: 810-813.

22. Lange, W. 1984. Piperonyl butoxide: synergistic effects on different neem seed extracts and influence on degradation of an enriched extract by ultra-violet light. Proc. 2nd Int. Neem Conf., Rauischholzhausen, pp. 129-140.

23. Tschesche, R. and Wulff, G. 1965 Uber die antimikrobielle Wirksamkeit von Saponinen. 2. Naturforsch. 20B: 543-546.

24. Kawasaki, T., Nishioka, T., Yamauchi, T., Miyahara, K. and Enbutsu, M. 1965. Digitalis saponins 111. Enzymatic hydrolysis of leaf saponins of *Digitalis purpurea* L. Chem. Pharm. Bull. 13: 435-440.

25. Hassaland, C. H. and Smith, B. S. W. 1957. Hecogenin from Agave sisalena by microbiological hydrolysis. Chem. & Industr. Pharm. 1570.

26. Yosiok, I., Fujio, M., Osamura, M. and Kitagawa, I. 1966. A novel cleavage method of saponin with bacteria intending to the genuine sapogenin on Senega and *Parax saponins*. Tetrahedron Lett. 50: 6303-6308.

27. FAO/WHO. 1973. Energy and Protein Requirements. FAO nutrition meeting report series, No. 52 Rome.

28. Starton, W.R. 1969. grain legumes in Africa, FAO, Rome, pp. 21-23.

29. Smart, J. 1976. Tropical Pulses. In: Tropical Agric., Series, Vol. 1: 7-9.

30. Odunfa, S. A. 1982. carbohydrate changes in fermenting African locust bean seed. J. plant Foods & Human Nutr. P. 310.

31. Sankaram, A.V.B., Marhanda, M. M., Bhaskaraiah, K., Subramanyam, M., Sultana, N., Sharma, H. C., Leuschner, K., Ramaprasad, G., Sitaramaiah, S., Rukmini, C. and Udayasekhara, P. R. 1986. Chemistry, biological activity and utilization aspects of some promising neem extracts. Proc. 3rd Int. Neem Conf., Nairobi pp. 127-148.

32. Liener, I. E. 1969. In: Toxic Constituents of Plant Foodstuffs. 2nd Ed. New York Akad. Press. Pp. 420-450.

33. Ketkar, C. M. 1976. Utilization of neem (Azadirachta indica) and its by-products. Khadi & Village Industries Commission, Bombay, pp. 38 and 177.

34. Nath, K., Rajagopal, K. S. and Garg, A. K. 1983. Water-washed neem (A. indica) seed kernel cake as cattle feed. J. Agric. Sci. 101: 323-326.