# NITRATE AND NITRITE IN RUMINANTS' BROWSE LEAVES

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## ABSTRACT

**The Nitrate ($N0_3$) and Nitrite ($N0_2$) concentrations were determined in some 37 browse leaves usually fed to ruminants. Concentrations of nitrates obtained ranged from 0.00 to 2790 mg, 2050 mg and 1540 mg per 100 g DM respectively in the leaves of trees, shrubs and herbs/grass while those of nitrite were also from 0.00 to 840, 80 and 441 mg respectively per 100 g DM of leaves. $N0_2$ concetration was generally lower than $N0_3$ in most of the leaves analysed. Feeding trials with goats indicated average $N0_3$ intake of 5.25 to 415.75 mg/d/wkg $^{0.734}$ while the respective DM intake values were between 22.34±5.01 and 85.36±3.78 g/d/Wkg $^{0.734}$. Mortalities were not recorded all through the experimental period.**

**Key Words:** Nitrate; Nitrite; Browse Leaves; Goats.

## INTRODUCTION

Toxicants in feeds, on the average, possess anti-nutritional effects on livestock and therefore tend to reduce the degree of reliability on the qualities of a feeding-stuff as reflected in the table of chemical composition. Nitrates and nitrites are Non-Protein Nitrogen (NPN) substances occuring in plant materials. The nitrate "per se" may not be toxic to animals but may be reduced by bacteria to nitrites (Arkhipov, 1989) which are likely to cause poisoning through combination with haemoglobin of the blood (Wilson, 1949) thereby forming a brown pigment, methaemoglobin (Bradley *et al.* 1949) which is incapable of transporting oxygen to the body tissues (Weart, 1948). Nitrite toxicity can lead to "hay poisoning". Nitrite is less toxic if the feed also contains soluble carbohydrates.

The nitrate contents of grasses vary with species, variety, manuring, etc. and the amount present is related to the crude protein content (Mc Donald, *et al.* 1982). Yoon *et al* (1990) fed rye-grass with O.469% $N0_3$ to whethers and found no significant changes in blood haemoglobin concentration although there were wide variations in the amounts of methaemoglobin formed following feeding. Takruri and Humeid (1988), however, indicated nitrate concentrations of 29-6743 mg/kg in herbs and vegatables while Arkhipov (1989) indicated maximum limits of $N0_3$ and $N0_2$ in green herbage fed to poultry as 200 and 10 mg/kg respectively. Chronic nitrite toxicity inhibits growth, decreases appetite and causes restlessness in chickens (Arkhipov, 1989).

The need to produce ruminant animals at cheap costs while fully exploring their potentials has, among others, resulted in the use of such feeds as browse plants. The objectives of this study therefore hinge on the quantification of the nitrates and nitrites in some leafy browse species consumed by ruminant animals in Nigeria. The effects of these toxicants on some performance characteristics of ruminants were also looked at.

## MATERIALS AND METHODS

### Browse Leaves Collection and Preparation

Leaves of thirty seven different browse species usually consumed by ruminants in the humid and sub-humid parts of Nigeria and other parts of the tropics, where collected after an initial survey which revealed their use in livestock production. Cassava leaves were obtained from IITA, Ibadan. Other leaves were collected from the botanical garden, Ibadan and some others from Edo and Imo States. The leaves were dried in a forced-air draught oven for 3 days at 50 - 60$^0$C, ground to 2mm sieve size and later assayed for their nitrate, nitrite and nitrogen concentrations. Three of these browse plants were fed to goats but these were

only dried at 50 - 60$^0$C and were not ground at all.

### Diets and Animals

Dried leaves of *Manihot sp., Spondias sp.* and *Gliricidia sp.* were fed to 20 West African dwarf (Fouta djallon) goats at 0, 25, 50, 75 and 100% levels in combination with *Manihot sp.* peels. Selection was based on their chemical potentials and availability in the Southern part of Nigeria in farmsteds and as fencing material but browsed by goats. The diets were allocated randomly to the five groups of goats which were allowed a maximum of 800 g of leaves twice daily at 08.00 and 16.00 hours. They had free access to fresh clean water daily and mineralized salt lick. Each trial lasted 21 days - 14 days for preliminary feeding and 7 days for collection of urine and faeces. The goats were balanced for sex and weight in a randomized block design.

### Analytical Methods

AOAC (1984) methods were followed in the determination of crude protein and dry matter.

Nitrate and Nitrite concentrations were estimated with the method of Rider and Mellon (1946) as modified by Ranjhan and Krishna (1980) using Bray's indicator and read off at 520mu wavelength with a Zeiss spectrophotometer PM 2A.

### Statistical Analyses

The data obtained were statistically analysed using the methods stated in Steel and Torrie (1980).

## RESULTS AND DISCUSSION

The various concentrations of nitrates and nitrites found in the analysed browse tree leaves are shown in Table 1. The $NO_3$ concentrations range from trace levels to 2790 mg/100g leaves DM. Notably high values were recorded for *Gliricidia sepium, Baphia nitida* and *Albizia zygia* which were also high in crude protein contents. The former two belong to the Papilionaceae family.

The nitrite concentrations were relatively low in most species but highest in *Baphia sp.* and *Samanea sp.* and remarkably low in *Dialium sp., Elaeis sp.* and *Spondias sp.* The proportions of nitrite to nitrate obtained in the leaves of the tree species studied were fairly low and range from 0.00% in a number species to 177.27% in *Spondias sp.* The crude protein values (N x 6.25) in these tree leaves were averagely high except in very few species, and so are indicative of very high N levels. Table 2 shows the levels of these two toxicants in some shrubs leaves. Values obtained were relatively lower than those of tree species. The proportions of Nitrite to Nitrate were low while the crude protein values were average. Nitrite concs were generally lower than those of nitrates in the shrubs.

Up to 40% of the herbs species analysed had no detectable nitrate levels (Table 3) while the nitrite concentrations obtained were higher than those of shrub and tree leaves assayed. In particular, *Manihot sp.* (clone No. 51077) had strickingly high nitrite level among the *Manihot species.* The nitrite concn as a proportion of the nitrate was also relatively high.

The relatively lower $NO_2$ concentrations recorded in the browse species may have been generated during the processing or drying of the leaves since this facilitates the conversion of nitrates to nitrites by bacteria. Nitrites are not usual components of freshly harvested vegatables but accumulate as a result of post harvest storage (Phillips, 1968). This has implications in the utilization of fresh forage as well as green leaves and vegatables for humans and other non-ruminant animals. Nitrates are not directly toxic to healthy adults but when reduced to nitrites in the upper gastro-intestinal tract of human infants or microflora of human mouth result in a direct toxicity of nitrite (Sherman, 1947). This has also been implicated in the possible formation of carcinogenic - N-nitroso compounds when it reacts with secondary and tertiary amino compounds in the stomach (Schupan *et. al.* 1967). Methaemoglobin, which inhibits oxygen transport to body tissues, also results from the effect of nitrite (Weart, 1949). The concentrations of $NO_3$ and $NO_2$ recorded in this

study fall within the range obtained by Takruri and Humeid (1988). Those in excess could be reflective of the N- status of the soil, as there is a direct relationship between them (Arkhipov, 1989).

The nitrogen contents of all these browse leaves ranged from 376 mg - 4945.6 mg/100g DM The results of the feeding trials with Manihot, Spondias and Gliricidia species are shown Table 4. No incidence of mortality was recorded all through the experimental period. Average Nitrate intake levels ranged from Zero to 670.00± 212.25 mg/d/$w_{kg\ 0.734kg}$ and were more in the *Manihot* and *Gliricidia spp.* trials, especially the sole-browse (100% Br) treatments. Nitrogen balance was observed to improve with increasing levels of dry matter intake and increasing peels supplementation with leaves. Weights were lost by goats on 100% Browse treatments with relatively high nitrate and nitrite intake level. The weight changes were, however, not significant ($P > 0.05$) in the GRL trials.

Nitrate/nitrite toxicity symptoms of growth inhibition and restlessness (Arkhipov, 1989) were not observed in these trials and this could infer that ruminants are more tolerant to these toxicants than monogastric animals. In spite of the relatively high nitrate and nitrite intakes at the 25, 50 and 75% level of browse use, the anti-nutritional effects such as restlessness, decreased appetite and growth inhibition (Arkhipov, 1989) were reduced and this was possibly because of supplemental energy (G.E. of 3.90 Kcal/g) from the *Manihot sp.* (Cassava) peels. This is known to give soluble carbohydrates. This stage of the study did not, however, involve the assessment of methaemoglobin levels of Yoon *et al* (1989). The weight lossess by goats on the 100% Browse trials may have arisen from their inability to meet their energy requirements from browse alone (Onwuka and Akinsoyinu, 1989) as this was observed to improve with increasing levels of *Manihot sp.* peels supplementation. These weight changes were, however, not significant for the animals on *Gliricidia sp.* trials. The essence of this study was to determine the qualities of dried browse plants in respect of the contents of the nitrates and nitrites.

## CONCLUSION

Nitrates and nitrites are present in plant leaves with nitrates levels generally higher. Plants' crude protein levels are also high. Small ruminants tend to be tolerant to the presence of Nitrates and Nitrites in dried green feed within the limits recorded in this study.

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**Table 3 NITRATE, NITRITE AND CRUDE PROTEIN CONTENTS OF [illegible] AND GRASS LEAVES**

| Names of the browse species | Family | Nitrates (mg/100 g DM) | Nitrite (mg/100 g DM) | [illegible] % of N's | Crude Protein (%) | Gross Energy (Kcal/g) |
|---|---|---|---|---|---|---|
| **Herbs** | | | | | | |
| Aframomum melegueta (K. Schum.) | Zingiberaceae | 800 | 22 | 2.75 | 14.30 | |
| Costus afer (ker-Gawl) | Zingiberaceae | - | 45 | 0.00 | 18.70 | |
| Manihot esculenta crantz No. 30211 | Euphorbiaceae | - | 441 | 0.00 | 28.04 | 4.57 |
| Manihot esculenta crantz " " 60337 | Euphorbiaceae | - | - | 0.00 | 22.71 | |
| M. esculenta " " 40764 | Euphorbiaceae | 870 | 115 | 13.22 | 20.42 | |
| M. esculenta " " 51077 | Euphorbiaceae | 420 | 401 | 95.48 | 26.55 | |
| M. esculenta " " 60506 | Euphorbiaceae | - | - | 0.00 | 24.97 | |
| Maratochloa leucantha K. Schum. | Marantaceae | - | - | 0.00 | 2.35 | |
| Musa cultivar A B (plantain) | Musaceae | - | - | 0.00 | 9.99 | |
| Palisota hirsuta K. Schum. | Comelinaceae | 1.540 | 80 | 5.20 | 16.91 | |
| Mean (Standard Brror) | | 363.00± 170.85 | 110.40± 53.29 | | 18.49±2.52 | |
| **Grass** | | | | | | |
| Bambusa vulgaris Schrad. ex Wendland | Gramineae | 90 | 160 | | | |



**Table 4 AVERAGE VALUES OF NITRATE AND NITRITE INTAKES, AND PERFORMANCE DATA OF WEST AFRICAN DWARF GOATS FED BROWSE LEAVES (BR) AND MANIHOT SP. PEELS (CAP) AS SUPPLEMENT.**

| | | 100% CAP (CONTROL) | 25% BR 75% CAP | 50% BR 50% CAP | 75% BR 25% CAP | 100% Br |
|---|---|---|---|---|---|---|
| Nitrate Intake ($mg/d/w_{kg}^{0.734}$) | CAL | $18.50\pm1.50^{a}$ | $150.00\pm7.07^{b}$ | $242.50\pm25.29^{c}$ | $332.50\pm26.50^{de}$ | $375.00\pm17.08^{e}$ |
| | SML | $1850.\pm1.50$ | $17.50\pm2.50$ | [illegible] | $8.50\pm1.50$ | $0.00\pm0.00$ |
| | GRL | $18.50\pm1.50^{a}$ | $475.00\pm123.86^{abc}$ | [illegible] | $620.00\pm226.31^{bc}$ | $670.00\pm212.25^{c}$ |
| Nitrite Intake ($mg/d/W_{kg}^{0.734}$) | CAL | $5.25\pm1.60^{a}$ | $135.00\pm6.46^{b}$ | [illegible] | $370.00\pm23.45^{de}$ | $385.00\pm17.08^{e}$ |
| | SML | $5.25\pm1.60^{a}$ | $13.75\pm2.14^{bce}$ | $20.50\pm3.69^{c}$ | $30.00\pm0.00^{d}$ | $12.50\pm2.50^{e}$ |
| | GRL | $5.25\pm1.60^{a}$ | $65.00\pm15.55^{a}$ | [illegible] | $415.75\pm385.82^{a}$ | $87.50\pm28.40^{a}$ |
| Dry matter intake ($g\ d^{-1}\ W_{kg}^{-0.734}$) | CAL | $42.20\pm5.60^{a}$ | $84.31\pm3.74^{b}$ | $75.44\pm5.44^{b}$ | $82.19\pm6.36^{b}$ | $85.36\pm3.78^{b}$ |
| | SML | $42.20\pm5.60^{a}$ | $59.72\pm6.21^{bcd}$ | $63.63\pm6.97^{cd}$ | $69.86\pm5.71^{d}$ | $22.34\pm5.01^{e}$ |
| | GRL | $42.20\pm5.60^{ac}$ | $64.28\pm4.12^{b}$ | $35.09\pm5.68^{a}$ | $31.72\pm10.23^{a}$ | $24.08\pm7.61^{a}$ |
| Body weight changes ($kg\ d^{-1}$) | CAL | $-0.13\pm0.6^{a}$ | $0.10\pm0.02^{b}$ | $0.14\pm0.02^{b}$ | $0.17\pm0.05^{b}$ | $-0.03\pm0.03^{a}$ |
| | SML | $-0.13\pm0.06^{a}$ | $0.04\pm0.04^{bc}$ | $0.06\pm0.02^{b}$ | $0.03\pm0.03^{bc}$ | $-0.06\pm0.02^{ac}$ |
| | GRL | $-0.013\pm.06$ | $0.07\pm0.02$ | $0.01\pm0.03$ | $0.06\pm0.08$ | $0.02\pm0.12$ |
| Nitrogen balance ($kg\ d^{-1}$) | CAL | $-0.07\pm0.03^{a}$ | $0.81\pm0.03^{b}$ | $1.40\pm0.15^{cd}$ | $1.77\pm0.14^{de}$ | $2.11\pm0.18^{e}$ |
| | SML | $-0.07\pm0.03^{ad}$ | $-0.05\pm0.09^{ad}$ | $0.20\pm0.02^{b}$ | $0.53\pm0.09^{c}$ | $-0.12\pm.09^{d}$ |
| | GRL | $-0.07\pm0.03^{a}$ | $0.23\pm0.14^{ab}$ | [illegible] | $0.39\pm0.21^{b}$ | $0.29\pm0.08^{ab}$ |

Means in the same row with different letters are significantly different ($P < 0.05$)
CAL = Manihot sp., SML = Spondias sp. and GRL = Gliricidia sp. leaves fed as browse (Br)
Figures are means ± Standard error

**Table 1 NITRATE, NITRITE AND CRUDE PROTEIN LEVELS IN SOME NIGERIAN TREES LEAVES FED TO LIVESTOCK**

| Names of the browse species | Family | Nitrates (mg/100 g DM) | Nitrite (mg/100 g DM) | $NO_2$ as % Of $NO_3$ | Crude Protein (%) | Gross Energy (Kcal/g) |
|---|---|---|---|---|---|---|
| Acioa barterii Hook F. & Olive Engl. | Rosaceae | 180 | 20 | 11.11% | 9.38 | |
| Albizia adianthifolia (Schum) W.F. Wight | Mimosacea | - | - | 0.00 | 23.30 | |
| Albizia zygia(DC). J.F. Macabr | Mimosaceae | 1,320 | - | 0.00 | 24.21 | |
| Baphia nitida Lodd. | Papilionaceae | 1,410 | 840 | 59.57 | 26.80 | |
| Cassia siamea Lam | Caesalpineaceae | 870 | 115 | 13.22 | 14.12 | |
| Ceiba pentandra Gaertn | Bombacaceae | 380 | 17 | 4.47 | 17.70 | |
| Crysophyllum albidium G.Don | Sapotacea | - | - | 0.00 | 14.30 | |
| Dialium guineesis Willd. | Caesalpineaceae | - | 15 | 0.00 | 12.76 | |
| Elaeis quineensis Jacq. | Palmaceae | 90 | 50 | 55.56 | 5.80 | |
| Gliricidia sepium (Jacq.) Staud | Papilionaceae | 2,790 | 360 | 12.90 | 20.69 | 4.35 |
| Musanga cecropioidea R.Br. | Moraceae | 390 | - | 0.00 | 12.31 | |
| Naudea diderrichii (L) De Willd. | Rubiaceae | 940 | 550 | 58.51 | 15.24 | |
| Parinari Korstingii (Engl.) | Chrysobalaneceae | 390 | 310 | 79.49 | 17.91 | |
| Parkia clappertoniana Kaey | Mimosaceae | - | - | 0.00 | 17.13 | |
| Samanea saman Merill | Mimosaceae | 440 | 780 | 177.27 | 7.50 | |
| Spondias mombin Linn. | Anrcardiaceae | - | 50 | 0.00 | 17.84 | 4.36 |
| Terminalia catappa Linn. | Combretaceae | - | 294 | 0.00 | 12.71 | |
| Mean (Standard error) | | 541.18 ± ± 180.47 | 200.06 ± 68.26 | | 15.87 ± 1.40 | |

**Table 2 NITRATE, NITRITE AND CRUDE PROTEIN CONTENTS OF SOME SHRUBS LEAVES IN NIGERIA**

| Names of the browse species | Family | Nitrates (mg/100 g DM) | Nitrite (mg/100 g DM) | $N^{\circ}_2$ as % Of $N^{\circ}_3$ | Crude Protein (%) |
|---|---|---|---|---|---|
| Alchornea cordifolia (Schum & Thron) mill | Euphorbiaceae | 550 | - | 0.00 | 20.57 |
| Alchornea sp. | Euphorbiaceae | 90 | - | 0.00 | 13.85 |
| Bridelia ferruginea (Benth) | Euphorbiaceae | - | 21.0 | 0.00 | 15.57 |
| Cnestis Ferruginea (DC) | Connaraceae | 360 | 80 | 22.22 | 19.37 |
| Codiaeum variegatum (Linn.) Blume | Euphorbiaceae | - | - | 0.00 | 12.95 |
| Cola milenii (K. Schum.) | Sterculiaceae | 90 | 8 | 8.89 | 20.98 |
| Combretum paniculatum (Vent.) | Combretaceae | 1,010 | - | 0.00 | 30.91 |
| Corpolobia lutea (G.Don) | Polygataceae | 2,050 | - | 0.00 | 4.70 |
| Microsdesmis puberula (Hook. F. & M. Zenikei) | Euphorbiaceae | 270 | 17 | 6.30 | 23.42 |
| Mean ± Standard Error. | | 491.11 ± 222.77 | 14.00 ± 8.68 | | 18.04 ± 2.47 |