

The formation of volatile N-nitrosamines in kikuyu grass silage

J.P. Marais

Biochemistry Section, Cedara Agricultural Research Institute, Private Bag X9059, Pietermaritzburg 3200, Republic of South Africa

Kikuyu grass (*Pennisetum clandestinum*) often contains high levels of nitrate and other NPN compounds. During silage fermentation it is likely that nitrosamines are formed which are well known carcinogens. An investigation on kikuyu silage confirmed the presence of at least two nitrosamines while similar analyses performed on maize silage were negative.

Kikoejoegras (*Pennisetum clandestinum*) bevat dikwels hoë vlakke van nitraat en ander NPN verbindings. Tydens kuilvoerfermentasie is dit moontlik dat nitrosamiene gevorm kan word, wat bekende karsinogene is. 'n Ondersoek van kikoejoekuilver het die teenwoordigheid van ten minste twee nitrosamiene bevestig, terwyl soortgelyke analises wat op mieliekuilver uitvoer is, negatief was.

Keywords: Kikuyu, *Pennisetum clandestinum*, silage, nitrosamines, milk

Introduction

Kikuyu (*Pennisetum clandestinum*) often comprises the bulk of the summer pasturage for milk production in the high rainfall areas of Natal. The highest fodder production from kikuyu pastures is obtained in December, January and early February and a surplus is often produced during this period which could be conserved for use during the winter. Owing to the high summer rainfall, hay production from kikuyu is impracticable and attempts have therefore been made to conserve kikuyu in the form of silage.

Kikuyu grass often contains high levels of nitrate and other non-protein-N compounds. During silage fermentation nitrate is reduced to nitrite which could react with secondary amines at low pH values to form nitrosamines. Nitrosamines are well known carcinogens which may be absorbed into the blood of the ruminant and transferred to the milk (Juszkiewicz & Kowalski, 1974).

This investigation was undertaken to establish whether nitrosamines are produced in kikuyu silage.

Methods

Kikuyu grass at an eight-week growth stage was cut, wilted, precision chopped and ensiled in a bunker silo at a dry matter content of 30% without any additives. After fermentation a silage sample was macerated in a Waring blender in aqueous solution. The fibrous material was filtered off and lead acetate and sulphamic acid were added to the extract to prevent the formation of artifacts (Van Broekhoven & Davies, 1980). The extract was vacuum distilled at 60°C and the distillate extracted with dichloromethane (Telling, Bryce & Althorpe, 1971). The dichloromethane phase was dried with anhydrous sodium sulphate and evaporated to a small volume. One cm³ of hexane was added as 'keeper solvent'. The extraction procedure was performed in the dark to prevent photo-decomposition of nitrosamines.

The extracted compounds were separated on silica gel G thin-layer plates and silica gel plates without binder but containing a UV indicator, using hexane-diethyl ether-dichloromethane (4:3:2) as solvent. Spots on the chromatograms were revealed by (i) the diphenylamine-palladium (II) chloride spray (Preussmann, Daiber & Hengy, 1964) followed by UV irradiation, (ii) the ninhydrin spray reagent and (iii) by observations under UV light.

Results and Discussions

The diphenylamine-palladium (II) chloride spray revealed one purple spot (Rf 0,83) on the silica gel G plate, indicating the presence of a nitrosamine. This compound is unstable if irradiated with short wave UV light and characteristically produces a brown spot on an unsprayed plate. It also

forms a brown spot if heated with a ninhydrin spray which indicates the presence of amino groups.

Under UV light four spots (Rf values 0,07, 0,54, 0,60 and 0,69) were visible on the silica gel plates containing the UV indicator. The two spots with Rf 0,54 and 0,60 gave positive reactions with the diphenylamine-palladium (II) chloride spray, while only the spot with an Rf of 0,69 gave a positive reaction with ninhydrin.

These results indicate that kikuyu silage contains at least two nitrosamines, while similar analyses for nitrosamines performed on maize silage gave negative results. Attempts are being made to identify and quantitate these nitrosamines using improved separation techniques.

References

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