THE SAFETY OF DIMETRIDAZOLE ALONE AND IN CONJUNCTION WITH OXYTETRACYCLINE IN HEREFORD CROSSBRED STEERS

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ABSTRACT

Dimetridazole was given intraruminally alone, and in conjunction with oxytetracycline to healthy, 10-11 month-old Hereford cross-bred steers (n = 6). Intraruminal treatment with dimetridazole was given through a fistula at 75 mg kg^{-1} daily for 5 d, while the oxytetracycline was injected intramuscularly at 10 mg kg^{-1} on Days 1 and 3 of the dimetridazole treatment. The animals were observed at various intervals throughout the trial period for adverse reactions, including effects on ruminal activity and motility, changes in live-mass, venous acid-base balance, haematology and ruminal and serum ammonia concentrations.

Dimetridazole, either when used alone or in conjunction with oxytetracycline, had a marked effect on ruminal function. Within 6 h of dosing, the ruminal pH fell to below 5, but then returned to pretreatment values over the next 24-48 h. This was followed by the eradication of the ruminal protozoal population in all animals tested and an increase in the methylene blue reduction time to more than 6 min. Ruminal motility remained unaffected throughout this period. During the week of treatment, the mean live-mass of the animals dropped by 20 ± 9,9 kg in the dimetridazole treated group and by 13,3 ± 2,8 kg in the animals treated with both dimetridazole and oxytetracycline. A mild to severe watery diarrhoea, which continued for 1 to 2 d, occurred in 4 animals after the first dimetridazole treatment. A compensated metabolic acidosis and an increase in haematocrit were observed. An initial transient rapid rise in rumen ammonia concentrations did not result in a concurrent rise in serum ammonia concentrations. Except for one animal, all the others recovered without intervention by the end of the trial period. However, in the one exception, it was necessary to administer fresh rumen content to re-establish ruminal activity. No significant differences were observed between the 2 treatment groups for any of the observations made.

Key words: Dimetridazole, oxytetracycline, safety, trichomoniasis, cattle, acid-base, rumen function.

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INTRODUCTION

The control of trichomoniasis in bulls has been achieved by therapeutic intervention[2]. Treatment is directed primarily towards infected bulls, since they represent the major source of transmission of the disease and also because the disease is regarded as self-limiting in cows[8]. Numerous topical treatments for trichomoniasis in bulls have been described[3 10]. These treatments are normally very elaborate, but are not always effective[9]. Systemic treatment, using various nitro-imidazole compounds such as metronidazole[8], ipronidazole[17] and dimetridazole[9 10], have been shown to be more effective. Metronidazole (Flagyl, Maybaker) appears to be less effective and is too costly for general use. Ipronidazole was previously the drug of choice, but has since been withdrawn from the market. Consequently the only systemically effective drug still available is dimetridazole. Dimetridazole has been administered per os to bulls at dosage rates of 50 to 100 mg kg^{-1}, daily for 5 d[1 4 11 12]. Most reports recommend the lower dosage rate. Dimetridazole dissolved in 20% sulphuric acid or dimethyl sulphoxide (DMSO) has been used for intravenous administration[16]. Numerous animals have been treated per os with no apparent adverse reactions, other than a mild indigestion, temporary reduction in appetite and a fall in milk yield[6]. The cause of these effects are not known, but has been ascribed to the effect of dimetridazole suppressing ruminal fermentation[6]. In the case of intravenous dimetridazole dissolved in 20% sulphuric acid, side-effects such as dyspnoea, ataxia, recumbency for up to 15 min and weakness for periods of up to 2 d, were noted[1]. These effects were transitory, but were regarded as unacceptable by the investigator.

Antimicrobial treatment, such as with oxytetracycline or penicillin, is given 1-2 d before dimetridazole treatment to reduce the numbers of preputial bacteria[17]. Preputial bacteria, particularly *Micrococcus spp,* are reported to cleave the imidazole ring of nitro-imidazole compounds in vivo and thereby reduce its efficacy. Without antibiotic pretreatment, efficacy of ipronidazole against trichimoniasis was reduced from 93% to 73%[17].

Recently, mortalities occurred in bulls following the use of dimetridazole (Emtryl base, Maybaker) at 75 mg kg^{-1} orally per day for 5 d (J Brandt 1989 Private Practitioner, Jan Kempdorp, personal communication). In one case, 8 out of 69 Hereford bulls died, while in another, 2 out of 110 Hereford cross bulls died. The animals exhibited nervous signs such as hyperexcitability, incoordination, chattering of the teeth and they eventually died. These effects appeared

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after the fourth treatment and progressed rapidly thereafter. Postmortem examination of 3 animals and histopathology of samples from one animal did not reveal any specific abnormality, except a rumen pH of 9 that was measured in one animal. Oxytetracyline HCl (Contromycin, Panvet) was administered at 20 mg kg^{-1} concurrently on Days 1 and 3 of the dimetridazole treatment.

Dimetridazole remains the only effective systemic drug available for the treatment of trichomoniasis in bulls. In the light of the unexplained mortalities, it was considered necessary that the safety of the product, particularly in combination with antibiotics, such as oxytetracycline be re-evaluated.

MATERIALS AND METHODS

Hereford crossbred steers (n=6), aged 10-11 months with a live-mass ranging from 202 to 288 kg, were used in the trial. The animals were purchased from a local feedlot which in turn had purchased them from a single owner in the eastern Cape Province. Forty eight days before the start of the trial, the animals were transferred to the research facilities at the Faculty of Veterinary Science, University of Pretoria. The animals were each identified by a numbered eartag.

The animals were kept together in a single open paddock. Each animal was fed 0,5 kg feed-concentrate per day in the morning and had ad libitum access to *Eragrostis* hay. Fresh borehole water was freely available in an automatic drinking bowl.

The animals were allocated to the treatment groups by restrictive randomisation, according to live-mass. Replicates of 2 animals each, one animal on each treatment, were formed after ranking by live-mass from the heaviest to the lightest animal. Allotment of the animals to replicates started from the heaviest animal and proceeded to the lightest animal. Within each replicate, the animals were randomly allocated to the treatment groups by means of a table of random numbers.

A rumen fistula, for the purpose of collecting rumen samples, was placed surgically in each animal on Day -39.

Each animal received one of the following treatments:

1. Dimetridazole at 75 mg kg^{-1} per day for 5 d.
2. Dimetridazole at 75 mg kg^{-1} per day for 5 d and oxytetracycline (Liquamycin 100, Pfizer) at 10 mg kg^{-1}, on Days 1 and 3. (Day 0 is the first day of dimetridazole treatment).

The animals were treated according to the live-mass measured on Day 0, after 12 h fasting and withholding of water. The dimetridazole was suspended in approximately 500 ml water and dosed through the rumen fistula. Oxytetracycline was administered intramuscularly in the neck. A maximum of 20 ml was administered per injection site.

Each animal was examined clinically, the measurements including heart rate, respiratory rate, rectal temperature and ruminal movements on Days -21, -7, -3, -1, 0, 1, 2, 3, 4, 5, 7, 10, 14 and 21. Live-mass was measured on Days -21, 0, 7, 14, 21 and 71. The animals were observed daily for adverse reactions.

Venous blood was collected from the jugular vein in heparinised 2 ml syringes for determination of blood pH and acid-base balance on Days -21, -14, -7, 0, 1, 2, 3, 4, 5, 7, 10, 14 and 21. The blood was kept on ice and the

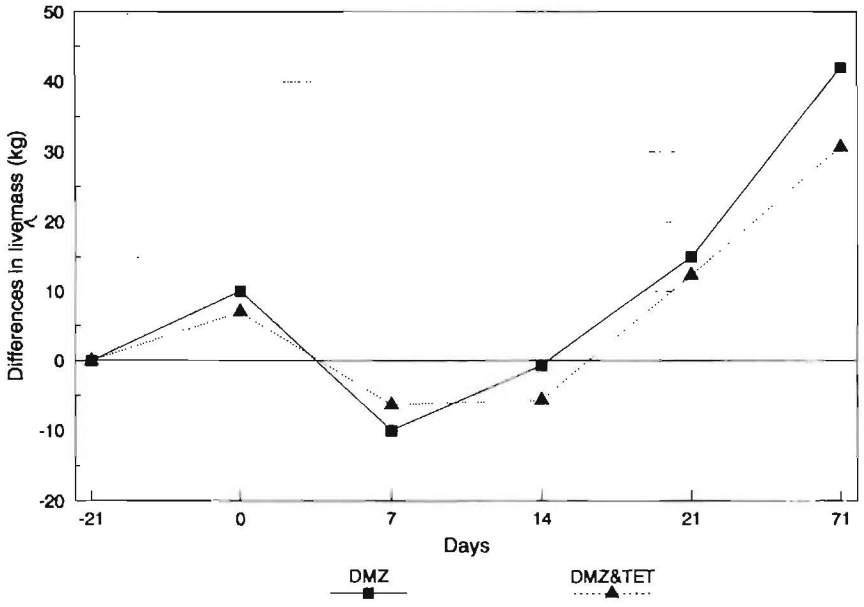

Fig. 1: **Changes in mean live-mass gain (kg) relative to individual live-mass at the start of the trial (Day-21) following treatment with either dimetridazole (DMZ) alone or in conjunction with oxy-tetracycline (DMZ&TET) in cattle**

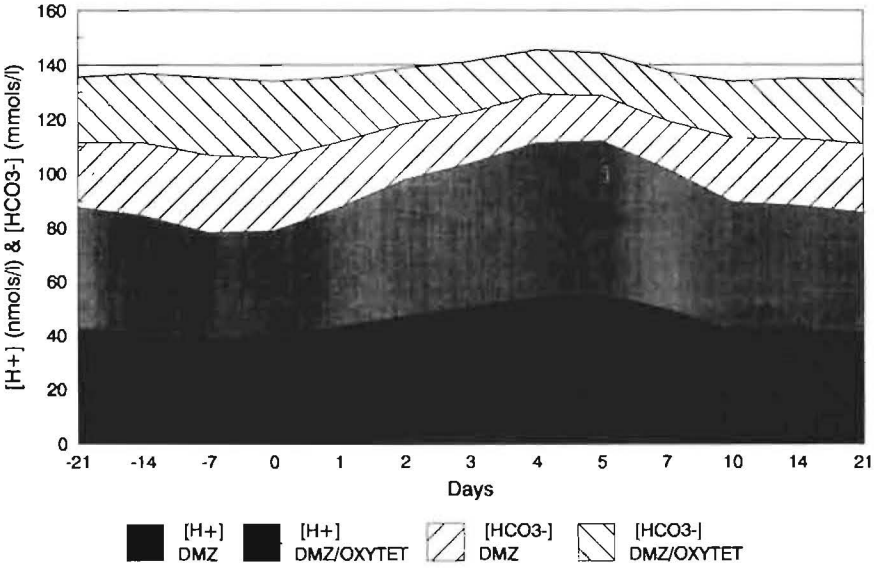

Fig. 2: **An area graph depicting changes in the mean venous hydrogen-ion (shaded areas) and bicarbonate-ion (hatched areas) concentrations before and after treatment with either dimetridazole (DMZ) alone or in conjunction with oxytetracycline (DMZ&OXYTET) in cattle**

pH, pCO_2 and pO_2 determined within one hour of collection on a bloodgas acid-base analyser (ABL3, Radiometer). Additional venous samples were collected in EDTA and plain vacuum tubes on Days -21, -14, -7, 0, 4, 7, 10, 14 and 21 for haematology and the determination of plasma Na^+, K^+ and Cl^-. Calcium concentrations were determined on blood collected on Days 0, 1, 2, 3, 4, 5 and 7. Sodium and potassium concentrations were determined by means of an ion sensitive electrode (Baker Analyte model + 1, Baker Instrument Corp.) directly on the sample, whereas chloride and calcium concentrations were determined on a RA1000-automated chemical analyser (Technicon Instrument Corp.).

Serum samples for the determination of blood enzymes ((aspartate transaminase (AST), creatine kinase (CK)), creatinine and ammonia were collected from each animal on Days -14, 0, 4, 14 and 21 and on Days 0, 1, 2, 3, 4, 5, 7, 10 and 14, respectively. Serum ammonia concentrations were determined immediately before and 2 h after treatment on Days 0, 1, 2 and 3. AST, CK and creatinine serum concentrations were determined using a RA1000-automated chemical analyser (Technicon Instrument Corp.). Ammonia was determined by a described method[5].

Evaluation of rumen activity included rumen pH, proteolytic activity using the methylene blue reduction test[7] and microscopic examination of rumen micro-organisms. These evaluations were done on Days -21, -14, -10, -7, 0, 1, 2, 3, 4, 5, 7, 10, 14 and 21. On Days 0, 1, 2 and 3 there were 2 examinations, one before and another 2 h after treatment. Rumen ammonia concentrations were determined at the same time as the serum ammonia concentrations, using the same method.

Treatment groups were compared by means of the Mann-Whitney test.

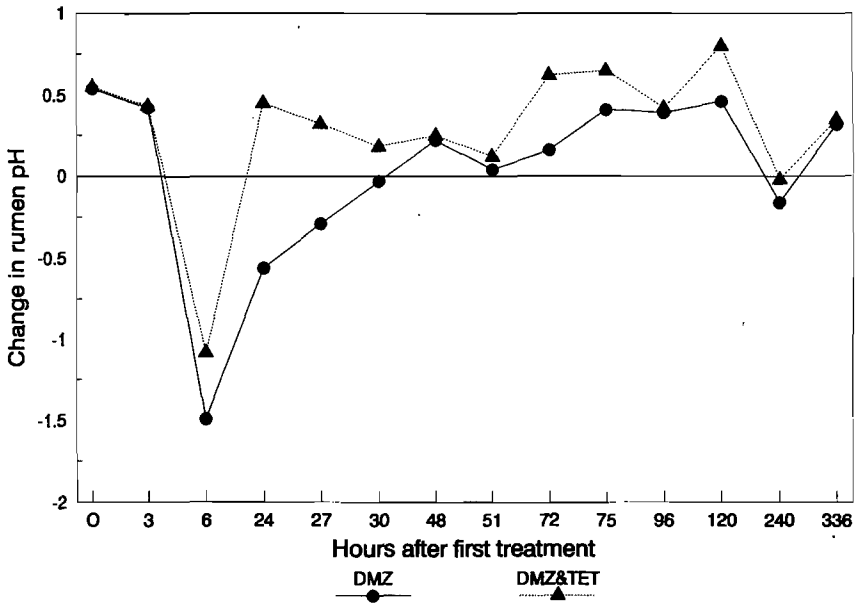

Fig. 3: **Mean rumen pH changes relative to the mean pretreatment rumen pH values following treatment with either dimetridazole (DMZ) alone or in conjunction with oxy-tetracycline (DMZ&TET) in cattle**

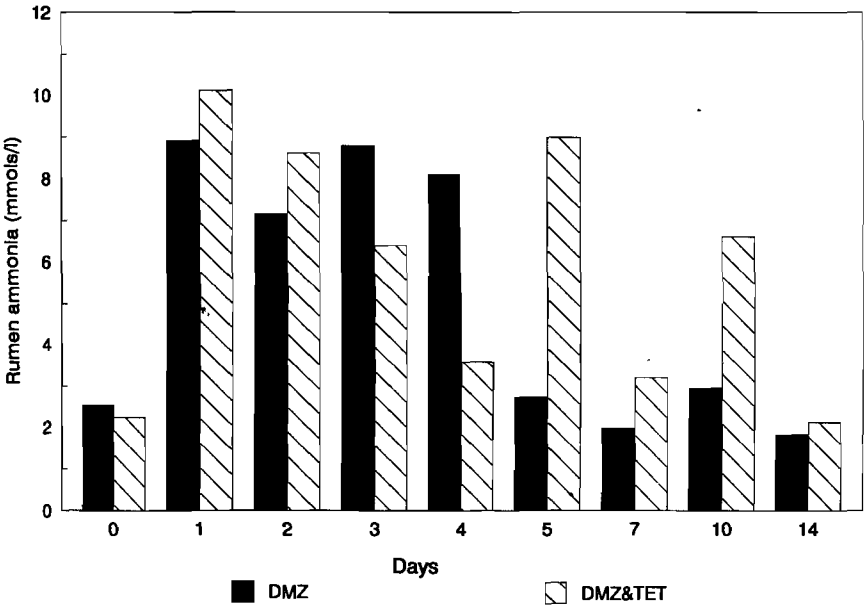

Fig. 4: **Changes in mean rumen ammonia concentration (mmols ℓ^{-1}) before and after treatment with either dimetridazole (DMZ) alone or in conjunction with oxytetracycline (DMZ&TET) in cattle**

RESULTS

A mild to severe watery diarrhoea occurred in 2 animals of each group 1-2 d after the first dimetridazole treatment and continued for 1 to 2 d. The rumen contents became dry and impacted 3 d after dimetridazole treatment. Most of these animals had recovered by Day 10, although, in 2 cases (one from each treatment group), 1-1,5 ℓ of water was added to the rumen contents at various intervals from Days 3 to 10 for the purpose of collecting samples. In addition, one animal had to be treated with fresh ruminal contents on Day 8, whereafter it started to recover.

Decreases in live-mass gains in both treatment groups following treatment are presented in Fig. 1. The differences in live-mass loss between the treatment groups were, however, not significant.

A mild to moderate metabolic acidosis developed during Days 1 to 5 (i.e. during and following the treatment period) (Table 1 and Fig. 2). These levels returned to normal over the next 2 weeks, but with the dimetridazole/-oxytetracycline treatment group lagging behind the dimetridazole group (although not significantly different). The venous pCO_2 mimicked the changes in mean venous bicarbonate concentrations.

The changes in mean rumen pH after treatment relative to the mean pretreatment values are illustrated in

Table 1: **Individual and mean (±standard deviation) venous pH recordings before and after treatment with either dimetridazole (DMZ) alone or in conjunction with oxytetracycline (DMZ&TET) in cattle**

Treatment group	Animal No.	Venous pH recordings before and after treatment (days) -21	-14	-7	0	1	2	3	4	5	7	10	14	21
DMZ	1	7,34	7,37	7,41	7,41	7,34	7,32	7,30	7,26	7,26	7,32	7,42	7,40	7,37
	4	7,39	7,41	7,41	7,41	7,37	7,34	7,32	7,32	7,30	7,32	7,39	7,40	7,39
	6	7,39	7,39	7,41	7,40	7,37	7,32	7,26	7,22	7,21	7,27	7,32	7,33	7,39
	Mean	7,37	7,39	7,41	7,41	7,36	7,33	7,29	7,27	7,26	7,30	7,38	7,38	7,38
	SD	0,02	0,02	0,00	0,00	0,01	0,01	0,02	0,04	0,04	0,02	0,04	0,03	0,01
DMZ&TET	2	7,32	7,43	7,41	7,42	7,38	7,36	7,32	7,30	7,34	7,36	7,40	7,40	7,33
	3	7,35	7,34	7,39	7,41	7,39	7,36	7,34	7,25	7,23	7,31	7,38	7,39	7,40
	5	7,37	7,40	7,42	7,38	7,31	7,19	7,19	7,19	7,19	7,20	7,22	7,24	7,34
	Mean	7,35	7,36	7,41	7,40	7,36	7,30	7,28	7,25	7,25	7,29	7,33	7,34	7,36
	SD	0,02	0,03	0,01	0,02	0,04	0,08	0,07	0,04	0,06	0,07	0,08	0,07	0,03

DMZ-dimetridazole TET-oxytetracycline SD-standard deviation

Fig. 3. The ruminal pH of both groups of animals decreased sharply within the first 6 h of the first dimetridazole treatment and then rose back to pretreatment values over the next 24-48 h. In one animal treated with both dimetridazole and oxytetracycline, a ruminal alkalosis developed from Day 3 through to Day 5, but resolved itself thereafter.

The protozoal population was eliminated after the second dimetridazole treatment in all animals and only returned after Day 14. The time value of the methylene blue reduction test increased above the accepted 6 min interval by Day 2 and only returned to values less than 6 min by Day 14. Ruminal contractions were detectable throughout the trial period and were subjectively judged to be fairly consistent with regards to frequency, length of contraction and amplitude.

The rumen ammonia concentrations varied considerably over the experimental period. Despite this, the concentration apeared to rise sharply after the first treatment in both treatment groups (Fig. 4). However, further changes to ammonia concentrations in the rumen before and 2 h after treatment, were inconsistent. Serum ammonia concentrations remained low throughout the trial period.

Apart from an increase in haematocrit during the treatment period, no other abnormal changes in haematology, electrolyte concentrations or serum enzymes were observed throughout the trial period.

DISCUSSION

The adverse effects that occurred after the administration of dimetridazole alone or in conjunction with oxytetracycline in this trial, were in all probability caused by the suppression of rumen microbial activity and were not due to any direct systemically-toxic effect of the drugs per se. According to Owens & Goetsch[13] inadequately-controlled microbial fermentation may be reflected by the incidence of bloat, ammonia toxicity, nitrate toxicity and acidosis.

Nitro-imidazoles are bactericidal to most gram-negative and many gram-positive anaerobic bacteria[15]. They become effective after entry into a bacterial cell and reduction of the nitro-group to a number of unstable intermediates, including antibacterial active metabolites[14]. Reduction takes place under anaerobic conditions in the presence of a low redox potential such as that found in the rumen. Consequently, it may be expected that high concentrations of active, intermediate metabolites will be formed. The most susceptible rumen microbes are generally the gram-negative lactolytic anaerobic organisms. Suppression of these organisms following the administration of dimetridazole, will result in the accumulation of lactic acid as the predominant acid end-product. This could explain the initial development of a ruminal acidosis. Progression of the antibacterial action per se, or as a result of further dimetridazole administration, could result in suppression of a broader spectrum of the ruminal microbes, thereby inhibiting the production of the volatile fatty acids, which will lead to a rise in ruminal pH. The decrease in the redox potential of the rumen results from the suppression of bacteria that normally scavenge available ruminal O_2 to maintain the rumen in a reduced state. Most bacteria in the rumen are obligatory anaerobes and therefore find O_2 toxic[13].

The apparent elimination of ruminal protozoa by dimetridazole, may not in itself result in any detrimental effects on animal health. Studies conducted with defaunated animals, either by chemical or by dietary change, showed that animals were not adversely affected[18]. However, the destruction of these organisms could have resulted in the initial sharp rise in ruminal ammonia concentrations.

The systemic metabolic acidosis that developed, is believed to have resulted from the ruminal acidosis that occurred. This metabolic acidosis would have been aggravated by the diarrhoea. Adequate respiratory compensation prevented any further serious systemic effects.

Oxytetracycline administered parenterally can result in the disturbance of ruminal flora. However, since no significant differences were observed between the two treatment groups, it does not appear that oxytetracycline contributed to the adverse effects in this case. In our opinion, the population was too small to make any valid comparison. It is also possible that the oxytetracycline dose administered in this trial was too low in comparison to the dose administered to the animals that had died in the field. Further research would be needed to clarify these aspects.

Although the adverse effects observed in this trial did not result in any mortality, they were serious enough to have jeopardised the lives of these animals. Various possible contributing factors under field conditions, such as withholding water, concurrent dietary disturbance of rumen function and presence of disease, could have adversely tipped the scale and have resulted in death. Furthermore, it is possible that the adverse effects on the rumen flora would be different in animals that are kept under extensive conditions as a result of differences in the type of microbial populations.

More research is required to evaluate these different factors and to specifically examine the influence of dimetridazole on the ruminal flora.

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