

New Zealand Society of Animal Production online archive

This paper is from the New Zealand Society for Animal Production online archive. NZSAP holds a regular annual conference in June or July each year for the presentation of technical and applied topics in animal production. NZSAP plays an important role as a forum fostering research in all areas of animal production including production systems, nutrition, meat science, animal welfare, wool science, animal breeding and genetics.

An invitation is extended to all those involved in the field of animal production to apply for membership of the New Zealand Society of Animal Production at our website www.nzsap.org.nz

[View All Proceedings](#)

[Next Conference](#)

[Join NZSAP](#)

The New Zealand Society of Animal Production in publishing the conference proceedings is engaged in disseminating information, not rendering professional advice or services. The views expressed herein do not necessarily represent the views of the New Zealand Society of Animal Production and the New Zealand Society of Animal Production expressly disclaims any form of liability with respect to anything done or omitted to be done in reliance upon the contents of these proceedings.

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](http://creativecommons.org/licenses/by-nc-nd/4.0/).



You are free to:

Share— copy and redistribute the material in any medium or format

Under the following terms:

Attribution — You must give [appropriate credit](#), provide a link to the license, and [indicate if changes were made](#). You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

NonCommercial — You may not use the material for [commercial purposes](#).

NoDerivatives — If you [remix, transform, or build upon](#) the material, you may not distribute the modified material.

<http://creativecommons.org.nz/licences/licences-explained/>

Effect of antioxidants on the toxicity of the facial eczema toxin, sporidesmin, in sheep

R. MUNDAY, E. MANNS and P. H. MORTIMER

Ruakura Agricultural Research Centre
Ministry of Agriculture and Fisheries, Hamilton

ABSTRACT

Four antioxidants — butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α -tocopherol and propyl gallate — have been tested for their ability to ameliorate the harmful effects of the facial eczema toxin, sporidesmin, in sheep. A moderate decrease in sporidesmin-induced liver damage (39% and 35% respectively, based on gross pathological assessment of liver injury) was observed in sheep pretreated with BHT and BHA; smaller effects were recorded in animals receiving the other compounds.

The possibility of an additive effect between antioxidants and zinc has also been investigated. It was shown that marked protection against sporidesmin toxicity, significantly greater than that given by either prophylactic alone, was afforded by simultaneous administration of BHT and zinc.

Keywords Sporidesmin toxicity; superoxide radical; antioxidants

INTRODUCTION

We have previously suggested (Munday, 1982) that sporidesmin, the mycotoxin responsible for facial eczema in ruminants, exerts its toxic effects through generation of the oxygen free radical, superoxide. Superoxide radical, and the other 'active oxygen' species which may be derived from it (hydrogen peroxide, hydroxyl radical, singlet molecular oxygen), are known to be toxic in diverse biological systems and there is evidence that intracellular generation of 'active oxygen' is responsible for the pathological changes induced in animals by a number of toxic compounds (Kappus and Sies, 1981).

Sporidesmin generates superoxide radical via a cyclic reduction/autoxidation reaction involving the di-thiol 'reduced sporidesmin'. This reaction has been extensively studied *in vitro* (Munday, 1982), while more recent experiments have provided evidence that it may also occur in living cells: hydrogen peroxide (a relatively stable breakdown product of superoxide) was detected in erythrocytes exposed to sporidesmin; these cells also suffered oxidative damage characteristic of that induced by 'active oxygen' species (Munday, 1983 a).

If our hypothesis concerning the mechanism of action of sporidesmin is correct, its harmful effects would be ameliorated either by treatments which serve to prevent the generation of 'active oxygen' species or by agents which are able to destroy such species before they exert their deleterious effects upon the animal.

The autoxidation of thiols and hence their ability

to generate superoxide, is inhibited by certain metals which form stable complexes (mercaptides) with -SH groups. Zinc, which is known to protect against sporidesmin toxicity in the living animal (Towers *et al.*, 1975), is a mercaptide-forming metal: we have shown that salts of this metal are potent inhibitors of superoxide generation from sporidesmin both *in vitro* and intracellularly; the severity of the oxidative changes induced by the mycotoxin in erythrocytes was also decreased in the presence of zinc (Munday, 1983 a, b). Of the other mercaptide-forming metals investigated in these test systems, most were less effective than zinc in inhibiting superoxide production from sporidesmin; only mercury and cadmium (which, in view of their exceptional toxicity, cannot be considered as potential prophylactics) were significantly more active than zinc. These studies indicate a possible mechanism for the protection afforded by zinc salts against sporidesmin toxicity *in vivo* and suggest that no other metals of this type are likely to supersede zinc as a practicable means of controlling facial eczema.

In recent studies, the alternative approach toward facial eczema prophylaxis — destruction of free radicals generated from sporidesmin — has been investigated. Many compounds are known, some of which are used in foodstuffs as antioxidants, which rapidly and specifically react with free radicals to yield inert products. Compounds of this type have been shown to destroy superoxide generated from sporidesmin *in vitro* (Munday, 1982) and to protect epithelial cells in tissue culture against sporidesmin toxicity (Munday, 1980). Four antioxidants — buty-

lated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α -tocopherol and propyl gallate — have now been tested for their ability to ameliorate sporidesmin toxicity *in vivo*; the possibility of an additive effect between antioxidants and zinc has also been investigated. The results of these experiments are described in the present report.

EXPERIMENTAL AND RESULTS

Protection Against Sporidesmin Toxicity by Antioxidants

Perendale hogget sheep were randomly allocated to 5 treatment groups, each containing 15 animals.

BHT, BHA and α -tocopherol, dissolved in peanut oil, were administered by intraperitoneal injection on days 1 to 5 of the experiment at a uniform dose level of 400 μ mol/kg/d (total dose 2 mmol/kg). Propyl gallate, as a solution in 50% (v/v) propylene glycol/peanut oil was similarly injected at a dose level of 300 μ mol/kg/d (total dose 1.5 mmol/kg); this lower dose, compared with the other agents, was necessary since administration of greater amounts of propyl gallate led to localised tissue irritation. Control sheep received 5 daily injections of peanut oil alone at a volume equivalent to that given to the test animals.

On days 3, 4 and 5 of the experiment, all sheep were given sporidesmin dissolved in water, by oral intubation at a dose of 0.07 mg/kg/d (total dose 0.21 mg/kg).

At day 21 of the experiment blood samples were taken for estimation of serum gamma-glutamyl transpeptidase (GGT) activity. The animals were

slaughtered at day 35 and the degree of liver damage sustained was assessed and scored after gross examination.

The mean serum GGT activities and the mean liver damage scores are shown in Table 1.

TABLE 1 Effect of antioxidants on mean serum GGT activities and mean liver-damage scores in sheep challenged with sporidesmin (0.21 mg/kg).

Group	Mean serum GGT (U/l, at day 21)	Mean log _e GGT	Mean liver-damage score (at day 35)
Control	311	5.32	1.53
BHT	170	4.74	0.93
BHA	105	4.49	1.00
α -tocopherol	226	4.97	1.20
Propyl gallate	253	5.11	1.40
S.E. of difference between 2 means		0.33	0.42

Additive Effect of Zinc and BHT in Protection Against Sporidesmin Toxicity

Eight groups, each of 15 sheep, were employed in this experiment. Animals in groups 1 and 5 received no pretreatment; those in groups 2 and 6 were given 5 daily doses of 400 μ mol/kg BHT, in peanut oil, by intraperitoneal injection, while sheep in groups 3 and 7 received 5 daily doses of 380 μ mol/kg zinc sulphate heptahydrate (equivalent to 25 mg/kg Zn²⁺) in

TABLE 2 Effect of BHT and/or zinc on mean serum GGT activities and mean liver-damage scores in sheep challenged with sporidesmin (0.21 mg/kg or 0.315 mg/kg).

Group	Total dose of sporidesmin	Mean serum GGT (U/l, at day 21)	Mean log _e GGT	Mean liver-damage score (at day 35)
1. Control	0.21 mg/kg	185	4.68	1.33
2. BHT	0.21 mg/kg	86	4.23	0.80
3. Zinc	0.21 mg/kg	70	4.11	0.53
4. BHT + zinc	0.21 mg/kg	43	3.70	0.20
5. Control	0.315 mg/kg	245	5.29	1.87
6. BHT	0.315 mg/kg	120	4.36	0.87
7. Zinc	0.315 mg/kg	200	4.90	1.33
8. BHT + zinc	0.315 mg/kg	92	4.18	0.67
S.E. of difference between 2 means			0.30	0.35
Mean responses:				
No BHT-BHT		90	0.63***	0.63***
No zinc-zinc		58	0.41**	0.53**
High-low sporidesmin dose		68	0.50***	0.47**
S.E.			0.15	0.17

None of the 4 interactions approached statistical significance.

aqueous solution by oral intubation. Sheep in groups 4 and 8 received both BHT and zinc at the above dose levels.

On days 3, 4 and 5 of the experiment, animals in groups 1 to 4 were given sporidesmin orally at a dose of 0.07 mg/kg/d (total dose 0.21 mg/kg), while sheep in groups 5 to 8 received the mycotoxin at a dose of 0.105 mg/kg/d (total dose 0.315 mg/kg).

The mean serum GGT activities (at day 21) and the mean liver-damage scores (at slaughter, day 35) are shown in Table 2.

DISCUSSION

All 4 of the antioxidants tested gave some degree of protection to sheep against the toxic effects of sporidesmin, although in the first experiment none gave results which were statistically significant at the 5% level of confidence. That the apparently large differences between some groups failed to satisfy such statistical criteria is a reflection of the marked variation in susceptibility to sporidesmin intoxication among sheep, an observation consistently recorded in experiments of this type.

Of the compounds studied, the synthetic monophenolic antioxidants BHT and BHA were the most effective prophylactics against sporidesmin toxicity, decreasing the liver-damage scores by 39% and 35% respectively. The naturally-occurring antioxidant α -tocopherol, which is also a monophenol, was intermediate in activity, while the least effective was the triphenol, propyl gallate. Even allowing for the fact that the dose level of propyl gallate employed in this experiment was only three-quarters of that of the other antioxidants, its efficacy was much less than would have been expected from earlier experiments (Munday, 1982) in which it was shown to be much more effective than BHT and α -tocopherol in destroying superoxide generated from sporidesmin *in vitro*. It is conceivable that the additional hydroxyl groups of propyl gallate, by increasing hydrophilicity, may increase its effectiveness in the purely aqueous *in vitro* system while decreasing its efficacy in the mixed aqueous/lipid environment pertaining intracellularly.

The present results with BHT are in accord with a previous report (Mortimer *et al.*, 1978) of the ability of this substance to protect against sporidesmin toxicity *in vivo*. In the previous study, the prophylactic activity of BHT was attributed to its ability to stimulate the hepatic cytochrome P-450 dependent mixed function oxidase system, rather than to its antioxidant properties. However, subsequent investigations showing that compounds such as DDT and piperonyl butoxide, which are much more potent stimulators of the P-450 system than BHT, afford no protection against sporidesmin toxicity (P. H. Mortimer *et al.*, unpublished) argue against this

hypothesis and suggest that BHT, like the other compounds employed in the present study (which either inhibit or are without effect upon the P-450 system [Dashman and Kamm, 1979; Netter, 1980; Branen, 1975]) acts by virtue of its ability to destroy free radicals.

If, as suggested, zinc and antioxidants act by different mechanisms, the protection afforded by 1 prophylactic would augment that given by the other. Support for this concept is provided by the results of the second experiment. While both BHT and zinc afforded some protection against sporidesmin toxicity, a combination of both agents was more effective than either alone and substantial protection against the harmful effects of the mycotoxin was obtained.

The protective activity exhibited by antioxidants is of interest in providing further evidence for the involvement of free radicals in sporidesmin toxicity; the augmentation by antioxidants of the protection afforded by zinc could be of interest in facial eczema control. At present, however, the use of antioxidants in conjunction with zinc is not a feasible proposition; repetitive parenteral administration, as employed in the present experiments, is not practicable in the field and this problem must be overcome before antioxidants can be considered as practicable prophylactics.

ACKNOWLEDGEMENT

We wish to thank Miss I. Gravett for advice on statistical analyses.

REFERENCES

- Branen A. L. 1975. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *Journal of the American Oil Chemists' Society* 52: 59-63.
- Dashman T.; Kamm J. J. 1979. Effects of high doses of vitamin E on dimethylnitrosamine hepatotoxicity and drug metabolism in the rat. *Biochemical pharmacology* 28: 1485-1490.
- Kappus H.; Sies H. 1981. Toxic drug effects associated with oxygen metabolism: redox cycling and lipid peroxidation. *Experientia* 37: 1233-1241.
- Mortimer P. H.; Manns E.; Sissons C. H. 1978. Liver mixed-function oxidase enzyme stimulation. New Zealand Ministry of Agriculture and Fisheries, Agricultural Research Division annual report 1977/8: 69.
- Munday R. 1980. Amelioration of sporidesmin cytotoxicity *in vitro*. New Zealand Ministry of Agriculture and Fisheries, Agricultural Research Division annual report 1979/80: 100.
- Munday R. 1982. Studies on the mechanism of toxicity of the mycotoxin, sporidesmin. I. Generation of superoxide radical by sporidesmin. *Chemico-biological interactions* 41: 361-374.
- Munday R. 1983 a. Studies on the mechanism of toxicity of the mycotoxin, sporidesmin. II. Inhibition by metals of the generation of superoxide radical by sporidesmin. *Chemico-biological interactions* (in press).

- Munday R. 1983 b. Studies on the mechanism of toxicity of the mycotoxin, sporidesmin. III. Evidence for intracellular generation of superoxide radical by sporidesmin. *Chemico-biological interactions* (submitted).
- Netter K. J. 1980. Inhibition of oxidative drug metabolism in microsomes. *Pharmacology and therapeutics* 10: 515-535.
- Towers N. R.; Smith B. L.; Wright D. E.; Sinclair P. 1975. Preventing facial eczema by using zinc. *Proceedings of the Ruakura farmers' conference* 27: 57-61.