

# **Detecting self-medication by grazing sheep against gastrointestinal nematodes.**

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## **Abstract**

Medicated feed blocks (MFB, containing an anthelmintic) could be used to control gastrointestinal nematodes (GIN) in sheep to achieve voluntary targeted selective treatment (TST) or self-medication. This would have the advantage of reduced selection pressure for anthelmintic resistance and reduced labour associated with providing treatment. The dynamics of MFB intake by sheep in a grazing environment are largely unknown because current techniques are not suitable for measuring supplement intake in grazing animals and/or measuring intake over a prolonged period.

The experiments in this thesis were designed for two purposes. Firstly, to develop a technique that met the requirements of a marker of MFB intake for use over an extended period in grazing livestock. Secondly, to use this technique to determine if an MFB could be used to achieve voluntary TST by establishing if grazing sheep display self-medication in response to GIN infection.

The methods that have been or could be used to estimate individual animal intake of supplements and evidence for and methods of detecting self-medication behavior were reviewed in Chapter 2. The characteristics of a suitable marker for measuring intake of medicated supplements in grazing sheep were outlined and fenbendazole (FBZ) and its metabolites were identified as being potentially suitable for this role.

The three experimental chapters described in this thesis have been submitted, and two published, as research articles to Animal Production Science. The abstract of each research article is provided below.

**Chapter 3:** This paper examines the potential of FBZ as a marker of intake. The following 5 experiments aim to determine the relationship between oral ingestion of FBZ and the plasma concentrations of FBZ and its metabolites oxfendazole (OFZ) and FBZ-sulfone (SUL) after single, multiple and daily doses both in housed and grazing sheep and sheep infected with GIN.

Factorial design was common across experiments where animals were given different dose rates at different frequencies with or without infection and the FBZ used was either powdered or incorporated into an unpressed block. The results from these experiments indicate that OFZ and SUL concentrations in plasma are dependent on FBZ dose rate in housed and grazing animals with significant differences evident between different dose rates ( $p < 0.001$ ). Variability of OFZ and SUL concentrations increase in grazing compared to housed animals. Area under the curve of metabolite concentrations was also shown to indicate dose rate regardless of the timing and frequency of dose. Step-wise regressions indicated that sampling every 48 h gave a good representation of area under the curve for different dose rates ( $R^2 = 0.951$ ,  $p < 0.001$ ). A significant separation of treatment means was achieved when samples were taken every 48 h and pooled during daily dosing with FBZ ( $p < 0.001$ ). Finally gastrointestinal nematode infection did not affect OFZ and SUL concentrations after daily doses of FBZ. The results from these experiments indicate that FBZ is a useful and accurate marker of supplement intake in grazing animals.

**Chapter 4:** The aim of this study was to determine the rate, variability and repeatability of intake by grazing sheep of a MFB containing FBZ and investigate if infection with GIN altered consumption patterns of the MFB in the same grazing mob. In Experiment 1, 30 Merino wethers were given access to a MFB for two separate one-week periods, with blood sampling at days 2, 4 and 6 of each period to determine MFB intake. In Experiment 2, 24 of the 30 wethers were selected based on previous MFB intake and allocated to receive an oral dose of 10,000 *Trichostrongylus colubriformis* and 3,000 *Haemonchus contortus* (anthelmintic susceptible) or a long acting anthelmintic. After five weeks, sheep were given access to an MFB (1.5 mg FBZ/g) and eight blood samples were taken over two weeks to determine intake. In Experiment 1, individual MFB intake in week 5 and week 7 was positively correlated ( $p = 0.002$ ,  $R^2 = 0.287$ ). Mean individual MFB intake in Experiment 1 and Experiment 2 was positively correlated ( $p = 0.008$ ,  $R^2 = 0.047$ ). In Experiment 2, more infected wethers (95%) ate the MFB than did uninfected wethers (79%) ( $p < 0.001$ ) and infected wethers ate significantly more MFB over the

first four days ( $p=0.041$ ) of access. Worm egg counts (WEC) in infected sheep declined from 2165 epg to 45 epg after access to the MFB. The decline in differences in MFB intake between infected and uninfected sheep corresponded to the decline in WEC, suggesting the existence of self-medication with parasitism accounting for intake differences.

**Chapter 5:** The aim of these experiments was to determine if self-medication, using an MFB containing an anthelmintic, was observed in two livestock classes. Two experiments were conducted concurrently with 60 lactating Merino ewes and 60 Merino wethers, respectively. Each experiment was split into 2 phases. In Phase 1 sheep were adapted to paddocks, familiarized with feed blocks and treatments were applied. Treatment was level of GIN infection ( $n=4$ ), sheep were infected with FBZ-susceptible *Haemonchus contortus* and *Trichostrongylus colubriformis* or given a long acting anthelmintic. In Phase 2, five weeks after infection, sheep were given access to a MFB ( $1.0 \pm 0.1$  mg FBZ/g) and nine blood samples taken over two weeks were analysed for FBZ metabolite concentration. Mean WEC at the start of Phase 2 was 1138 epg in infected ewes and 578 epg in infected wethers. At the end of Phase 2 efficacy of FBZ was 21% in ewes and 22% in wethers. A higher percentage of infected ewes (82%) consumed the MFB than uninfected ewes (60%) ( $p<0.001$ ). Of the ewes that consumed the MFB there was no difference in the amount consumed by infected and uninfected groups. There was no difference in the percentage of infected and uninfected wethers eating the MFB or in the amount they ate. There was a significant and negative relationship between WEC and MFB intake in ewe and wether experiments ( $p<0.050$ ). The ewe results indicate self-medication may take the form of stimulating exploration for food resources rather than MFB consumption. Self-medication was not observed in the wethers but lower worm burden indicated by WEC may not have provided enough stimulus to elicit a response.

The data collected for this thesis provides support for FBZ (in a role separate to its anthelmintic activity) as a useful and accurate marker of feed block intake in grazing sheep over (at least) a six day period. Self-medication using a MFB was demonstrated by sheep infected with anthelmintic susceptible GIN. In the final series of experiments the establishment of FBZ-resistant GIN meant

that intake of MFB was not curative. Nevertheless, GIN infection was seen to increase exploration for food, depending on severity of GIN infection. While a positive relationship between MFB consumption and severity of GIN infection was not confirmed, these results suggest that MFB may be a prospective tool for achieving voluntary TST.

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## List of abbreviations

CV	Co-efficient of variation
FBZ	Fenbendazole
GIN	Gastrointestinal nematodes
HPLC	High performance liquid chromatography
OFZ	Oxfendazole
MFB	Medicated feed block
ML	Macrocyclic lactones
SUL	Fenbendazole-sulfone
TST	Targeted selective treatment
WEC	Worm egg count

Note: Each of the experimental chapters has been submitted for publication as individual papers. For this reason abbreviations are re-stated when they are first used in each chapter. For the sake of readability not all abbreviations from the above list are used in every instance.

## List of Publications and Awards

Fishpool, FJ, Kahn, LP, Tucker, DJ, Nolan, JV, Leng, RA (2011) Fenbendazole as a possible marker of supplement intake in sheep. In 'Proceedings of the New Zealand Society of Animal Production. Invercargill, New Zealand'. (Ed. R Sumner) Volume 71 pp. 13-16. (New Zealand Society of Animal Production (Inc.)).

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