BRIEF COMMUNICATION: Chitosan is a highly effective in vitro antibacterial agent against the strains of bacteria causing footrot, but is not effective in treating stage-four footrot on farm.

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Introduction

Footrot affects sheep, deer, cattle and goats. It is caused by a mixed bacterial infection of Fusobacterium necrophorum and Dichelobacter nodosus. Footrot is highly contagious and spreads rapidly in warm and moist conditions. Virulent footrot results in the destruction of hoof tissue resulting in reduced animal weight, fertility and wool production. If untreated, footrot can lead to a slow and painful death (Kennan et al. 2011). Nationally, footrot is estimated to cost $80–100M pa in lost production (Greer, 2005; Hickford et al. 2005).

Chitin is an abundant waste product from the seafood industry that can be converted to biodegradable chitosan, a compound that has proven biomedical antimicrobial and haemostatic/wound healing properties (Vinsova & Vavrikova 2008). NZ has a unique abundance of chitin as a waste material from its fishing industry (squid pens and crab shells). By coupling this cheap source of raw material with minimal processing to derive chitosan, a cost-effective method to prevent and/or treat footrot compared to using vaccines, zinc sulphate footbaths and antibiotic treatment and without the trait trade-offs inherent with genetic-selection approaches is possible. We have previously reported research, using chitosan derived from snow crab, that demonstrated high in vitro antibacterial activity of chitosan derivatives against D. nodosus and F. necrophorum (Mros et al. 2012). The present study was aimed at investigating the efficacy of chitosan at higher concentrations for the treatment of footrot in merino sheep under normal farming conditions.

Materials and methods

Chitosan

Alaska snow crab was obtained from Waseta International (Shanghai, China). A chitosan derivative was prepared that had 75% deacetylation degree and was dissolved in 1% acetic acid to prepare a 10% (w/w) solution that was diluted with water for use on farm.

Bacterial strains and minimum inhibitory concentration assays

Strains of bacteria associated with the pathogenesis of footrot Dichelobacter nodosus (Wallaceville, AgResearch Centre) and Fusobacterium necrophorum (NCTC 10575, DSM 20698) Type A were grown as described earlier (Mros et al. 2012).
Statistical analysis

A student test (t-test) was used to test differences between the MIC values for chitosan and streptomycin sulphate. Analysis of variance (ANOVA) was carried out using Minitab (version 16.2.4). A general linear model was used to determine the effects of treatments on weight gain and footrot scores. Significant differences among mean values were determined at a 5% significance level.

Figure 1. Mean weight gain (mean ± SEM) of sheep with grade-four footrot randomly assigned to four experimental groups of ten animals each. The animals were foot bathed in either zinc sulphate, 1 mg/ml chitosan or 10 mg/ml chitosan solutions four times over the 24-day trial or left untreated. The sheep were weighed before and after 24 days of treatment.

![Weight gain graph](image1)

Figure 2. Footrot scores of sheep feet used in the present study. Results are expressed as mean score (± SEM) for footrot infection in sheep with grade-four footrot randomly assigned to four experimental groups of ten animals each before and after 4 days of treatment. The animals were foot bathed in either zinc sulphate, 1 mg/ml chitosan or 10 mg/ml chitosan solutions four times over the 24-day treatment period or left untreated. Pre = pre-treatment and post = post-treatment.

![Footrot score graph](image2)

Results

Antimicrobial activity of chitosan derivatives

The in vitro antimicrobial activity of the chitosan against the two bacterial strains involved in the pathogenesis of footrot, *D. nodosus* and *F. necrophorum* showed no growth on agar at a concentration of 0.0078 mg/ml and the positive control at 0.015mg/ml. Acetic acid (1%) did not inhibit growth of the bacteria.

Sheep treated with zinc sulphate had the highest weight gain (6.6 ± 0.69 kg) over the 24 days treatment period. This was significantly higher than for the sheep treated with 1 mg/ml of chitosan (P < 0.05). The average weights of sheep from untreated control and treated with 10 mg/ml chitosan solutions were not significantly different from either the zinc sulphate treated or the 1 mg/ml chitosan treated sheep (Figure 1). Only zinc sulphate treatment demonstrated any improvement in the treatment of footrot compared to the untreated group (Figure 2).
Discussion

Chitosan is a known antimicrobial agent effective against a wide range of organisms (Vinsova & Vavrikova 2008). We have previously reported the antimicrobial action of chitosan against the anaerobic organisms implicated in the pathogenesis of footrot (Mros et al. 2012) and the present study confirmed that activity using a freshly made chitosan preparation. The MICs of chitosan against the microorganisms that cause footrot were lower than for the therapeutic antibiotic streptomycin sulphate. Chitosan damages the cell membrane of D. nodusus causing a peripheral cytoplasm leakage that subsequently leads to the death of the microorganism (Mros et al. 2012). Under in vitro conditions, the damage can be observed within two hours of incubation of the chitosan with the organism. It is normal practice to increase the amount of a potentially therapeutic compound when taking it from an in vitro to an in vivo trial. In this instance amounts of 1 mg/ml and 10 mg/ml were chosen. However, these amounts were not high enough to have an effect on treating footrot in this trial even though the in vitro results were outstanding.

Environmental conditions (constant exposure to a wet contaminated environment) may have had an impact on this trial as well as running the untreated controls with the treated animals so that there was constant exposure to the microorganisms that cause footrot. However, this never-the-less mimics the necessary real-world performance conditions required for commercial application. Further on farm investigations by using either higher concentrations of chitosan, more frequent foot bathing or delivering the chitosan in a different formulation are required to determine how the high lab-based efficacy against footrot bacteria can be translated into high field-based efficacy.

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References

Eloff JN 1998. A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. Planta Medica 64: 711-713