

# Garlic impairs *Actinobacillus pleuropneumoniae* in vitro and alleviates pleuropneumonia in a pig model

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## Goal

A huge number of diverse sulfurous compounds have been identified in garlic preparations, and many of them are associated with health-supporting properties. Digestion products of sulfurous compounds from garlic are to a certain extent excreted via the lungs, such as allyl methyl sulfide (AMS), and could therefore have an effect on the course of pneumonia in pigs.

The objectives of this study were (i) to test the susceptibility of the pig pathogen *Actinobacillus pleuropneumoniae* to AMS in *in vitro* experiments, and (ii) to assess the impact of garlic on systemic blood AMS levels and on clinical and pathological symptoms in the lungs of pigs experimentally infected with *A. pleuropneumoniae*.

## Methods

(i) In *in vitro* experiments, the effect of AMS on the growth of *A. pleuropneumoniae* serotype 9 was examined in closed bottles equipped with a photometer tube. The bottles were incubated at 37°C and the growth of *A. pleuropneumoniae* was monitored as optical density at 600 nm.

(ii) In an *in vivo* challenge trial, 15 seven-week-old pigs, which received a diet with 5% of a commercial garlic feed component, and a control group of 15 pigs, which received a diet without garlic, were infected with *A. pleuropneumoniae* serotype 2 by exposure to an aerosol, and subsequently followed for 4 days. For AMS analysis, blood samples were taken at the day of experimental infection. At the end of the trial, the pigs were euthanized and gross pathology findings were recorded.

## Results and Discussion

(i) In the *in vitro* experiments, AMS was shown to exhibit an antibacterial effect against *A. pleuropneumoniae* serotype 9. At 1.1 mM, AMS impaired the growth rate of *A. pleuropneumoniae* by 8% compared to unimpeded growth (Fig 1). Although causing a delay in the growth of *A. pleuropneumoniae* when compared to unaffected growth in medium, AMS did not lower the stationary phase yield of *A. pleuropneumoniae*. The sensitivity to AMS was regarded as an indication that garlic with its plethora of decomposition products might have an effect on *A. pleuropneumoniae* in vivo.

(ii) In the *in vivo* challenge trial, blood AMS in the garlic-fed group amounted to  $0.32 \pm 0.13 \mu\text{M}$  at the day of the challenge, whereas in the control group no AMS was detected. At the end of the experiment, the occurrence of characteristic pleuropneumonia lesions in 47% of the lungs of the control group and in 27% of the lungs of the garlic-fed group, in combination with a near to significant ( $p = 0.06$ ) lower relative lung weight in the garlic-fed group, indicated a beneficial, alleviating effect of garlic on the course and severity of the *A. pleuropneumoniae* infection (Becker et al., 2012).

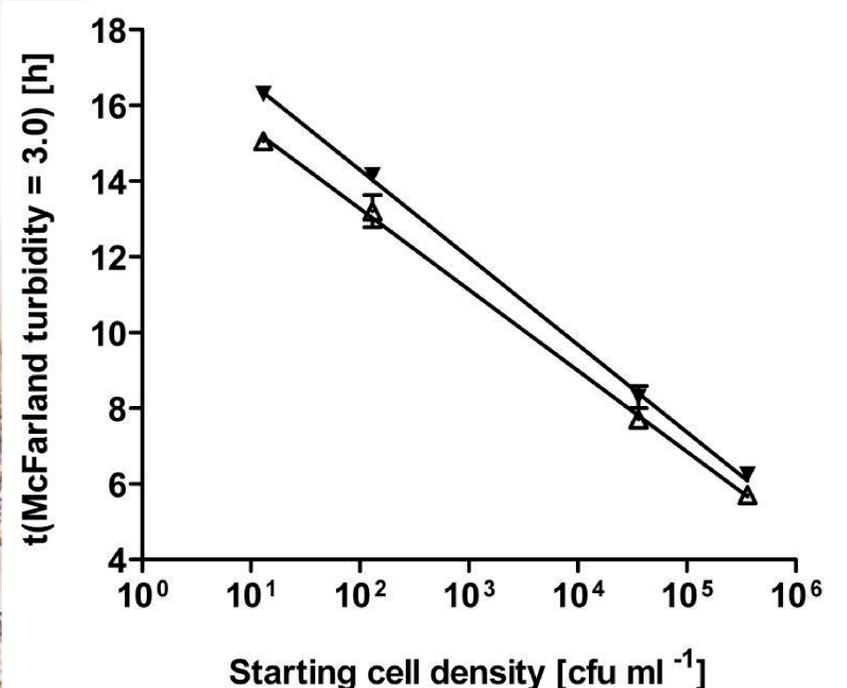


Fig. 1. Effect of AMS on the growth of *Actinobacillus pleuropneumoniae* type 9 (CVI 1.23) at different starting cell densities. The appearance of growth is depicted as time-coordinate at which a McFarland turbidity of 3.0 was reached:  $\Delta$ , 0.0 mM AMS;  $\blacktriangledown$ , 1.1 mM AMS in medium. The regression lines follow the equations  $y = -2.14 \log(x) + 17.55$  ( $R^2 = 0.999$ ) without AMS, and  $y = -2.29 \log(x) + 18.90$  ( $R^2 = 0.999$ ) with AMS. Error bars indicate standard deviations.

## Reference

Becker, P.M., van Wikselaar, P.G., Mul, M.F., Pol, A., Engel, B., Wijdenes, J.W., van der Peet-Schwering, C.M.C., Wisselink, H.J., Stockhofe-Zurwieden, N. (2012) *Actinobacillus pleuropneumoniae* is impaired by the garlic volatile allyl methyl sulfide (AMS) in vitro and in-feed garlic alleviates pleuropneumonia in a pig model. *Vet. Microbiol.* 154, 316–324.

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