

# Recombinant *Limosilactobacillus* (*Lactobacillus*) delivering nanobodies against *Clostridium perfringens* NetB and alpha toxin confers potential protection from necrotic enteritis

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## Abstract

Necrotic enteritis (NE), caused by *Clostridium perfringens*, is an intestinal disease with devastating economic losses to the poultry industry. NE is a complex disease and predisposing factors that compromise gut integrity are required to facilitate *C. perfringens* proliferation and toxin production. NE is also characterized by drastic shifts in gut microbiota; *C. perfringens* is negatively correlated with *Lactobacilli*. Vaccines are only partially effective against NE and antibiotics suffer from the concern of resistance development. These strategies address only some aspects of NE pathogenesis. Thus, there is an urgent need for alternative strategies that address multiple aspects of NE biology. Here, we developed *Limosilactobacillus* (*Lactobacillus*) *reuteri* vectors for in situ delivery of nanobodies against NetB and  $\alpha$  toxin, two key toxins associated with NE pathophysiology. We generated nanobodies and showed that these nanobodies neutralize NetB and  $\alpha$  toxin. We selected *L. reuteri* vector strains with intrinsic benefits and demonstrated that these strains inhibit *C. perfringens* and secrete over 130 metabolites, some of which play a key role in maintaining gut health. Recombinant *L. reuteri* strains efficiently secreted nanobodies and these nanobodies neutralized NetB. The recombinant strains were genetically and phenotypically stable over 480 generations and showed persistent colonization in chickens. A two-dose in ovo and drinking water administration of recombinant *L. reuteri* strains protected chickens from NE-associated mortality. These results provide proof-of-concept data for using *L. reuteri* as a live vector for delivery of nanobodies with broad applicability to other targets and highlight the potential synergistic effects of vector strains and nanobodies for addressing complex diseases such as NE.

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