In both segments, O-glycosylation of mucins was strongly affected, with the appearance of elongated polylactosaminic-chain containing O-glycan structures, associated with flattening and loss of the mucus layer cohesive properties specifically in the ileum. *L. farcininis* bound to intestinal Muc2 and prevented WAS-induced functional alterations and changes in mucin O-glycosylation and mucus physical properties.

**Conclusion:** WAS-induced functional changes were associated with mucus alterations resulting from a shift in O-glycosylation rather than from changes in mucin expression. *L. farcininis* treatment prevented these alterations, conferring epithelial and mucus barrier strengthening.

**Keywords:** gut permeability, mucus layer, water avoidance stress.