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Efficacy of probiotics as alternatives to antimicrobials

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Key Conclusions

- ***In vitro* testing demonstrated that probiotics are a valid alternative to the use of antibiotics to control some bacterial infections.**
- **CLOSTAT® is more efficacious *in vitro* at controlling selected pathogens than other probiotic competitors.**

INTRODUCTION

Antimicrobials have been a key tool used to fight against infectious diseases for as long as they are available.

In the livestock sector, antimicrobials can be used for therapeutic purposes (treatment of sick animals), prophylaxis (when antimicrobials are administered to a herd or flock of animals at risk of disease) or metaphylaxis (when antimicrobials are administered to clinically healthy animals in the same flock or group of animals with clinical signs). In the past, antimicrobials were also used for antimicrobial growth promotion (AGP), an application now banned in the EU (since 2006) and more pressure is mounting globally for this ban to extend to other markets and regions.

The use of antimicrobials as AGPs started due to the benefits they have on intestinal health, feed use efficiency and microbiome.

The objective of the trial was to determine the *in vitro* antibacterial activity, which may support the choice of probiotics as alternatives to metaphylactic and prophylactic antimicrobial use.

KEYWORDS

CLOSTAT®, bacteriostatic, antimicrobials, *Salmonella, Escherichia, Staphylococcus, Clostridium*

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MATERIAL AND METHODS

Four foodborne bacteria were tested, including *Salmonella enterica* subsp. *Typhimurium* (ATCC 14028), *S. aureus* (ATCC 25923 AT), *E. coli* (ATCC 35218 EC), and *C. perfringens* (ATCC 13124).

Three commercial probiotic strains were obtained in a freeze dried powder form, probiotics included CLOSTAT®, a unique strain of *Bacillus sp.* PB6 and two competitor probiotic products, one based on *Bacillus licheniformis* (PRO1) and a probiotic containing various strains of both *B. subtilis* and *B. licheniformis* (PRO2).

A colony overlay assay was used to demonstrate antibacterial activity. Overnight cultures of each probiotic strain were inoculated onto Luria-Bertani (LB) agar plates and incubated. After the development of probiotic colonies, plates were exposed to chloroform vapor and overlayed with LB and BHI (*Clostridium perfringens*) agar previously inoculated with each of the tested pathogen. All plates were then incubated aerobically or anaerobically (*C. perfringens* plates) at 37°C, for 48 h.

Inhibition zones surrounding the spots were measured in millimeters. Experiments were carried out twice in triplicate.

Cell-free Culture Supernatants (CFCS) from probiotic cultures were also tested, supernatants were harvested and used in disc diffusion assay. The diameter of the inhibition zone (DIZ) was measured in millimeters. Inhibition zones larger than 7 mm in diameter were considered positive.

RESULTS AND DISCUSSION

Salmonella typhimurium was susceptible to both probiotics, CLOSTAT and PRO1 but not to PRO2. *S. aureus* was sensitive to the three compounds, with only moderate effects observed for PRO2. *E. coli* was sensitive to the three probiotics. *C. perfringens* displayed a statistically significant susceptibility only to CLOSTAT followed by marginal inhibition with PRO2, no effect was observed with PRO1.With the CFCS, only the CLOSTAT supernatant resulted in growth inhibition of *C. perfringens*.

CLOSTAT consistently showed the highest efficacy across all tested bacteria, while both PRO1 and PRO2 showed statistically significant, but relatively lower effects ($P < 0.05$). A summary of the effect of the probiotics on the bacterial growth can be seen on Table 1.

Table 1. Effect of Probiotic against bacteria

	Salmonella typhimurium	*Escherichia coli*	*Staphylococcus aureus*	*Clostridium perfringens*
CLOSTAT	24.00 ±0.00^{a}	26.00 ±1.42^{a}	23.75 ±1.06^{a}	24.33 ±1.16^{a}
PRO1	23.83 ±0.76^{a}	24.00 ±1.41^{ab}	21.75 ±1.06^{a}	0.00 ±0.00^{b}
PRO2	0.00 ±0.00^{b}	19.00 ±1.41^{b}	15.50 ±0.71^{b}	9.83 ±6.64^{b}

different superscripts (ab) within a row indicate statistically significant difference at $P < 0.05$

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CLOSTAT showed inhibitory effects against *S. Typhimurium, E. coli, S. aureus,* and *C. perfringens*, the inhibitory effect was not due to competition for nutrients or competitive exclusion, as the agar plates were sterilized. Gram-positive bacteria were more sensitive to the tested probiotics than gram negative bacteria.

CONCLUSION

In this *in vitro* trial, CLOSTAT demonstrated that it inhibited the growth of important pathogens for animal production, *S. Typhimurium, E. coli, S. aureus,* and *C. perfringens,* more importantly, it was the only probiotic that had a clear inhibiting effect on the microbial growth. The antimicrobial effect of CLOSTAT is clearly direct, not relying on competitive exclusion.

This *in vitro* research demonstrates probiotics have the potential to control microbial pathogens and can be considered as alternatives to the use of antimicrobials.

REFERENCES

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