

Effects of Complementary Feed with Postbiotics Derived from *Bacillus subtilis* on Nutrient Digestibility, Faecal Characteristics, Gut Microbiota, Metabolic and Immune Responses, and Coat Quality in Healthy Senior Dogs

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The aim of this study was to evaluate the effects of a *Bacillus subtilis*-derived postbiotic on apparent total tract nutrient digestibility, faecal fermentation characteristics, gut microbiota, immune response, blood biochemistry, hematological parameters, serum trace minerals, and coat quality in healthy senior dogs. Twenty-one healthy senior Golden Retriever dogs (9 ± 1.0 years old) were randomly allocated to three dietary treatments for 28 days: a control diet (CON) or the same diet supplemented with 0.3 (POS1) or 0.6 mL/day (POS2) of a *Bacillus subtilis*-derived postbiotic. Apparent nutrient digestibility, faecal characteristics, blood variables, and coat quality were evaluated, whereas faecal bacterial communities were characterized by 16S rRNA gene amplicon sequencing, followed by taxonomic classification and alpha- and beta-diversity analyses. Postbiotic supplementation significantly improved crude protein and crude fiber digestibility while reducing faecal pH and ammonia concentrations and increasing acetate, propionate, and total short-chain fatty acid concentrations ($p < 0.05$). Alpha diversity was minimally affected, with a significant difference observed only for amplicon sequence variant (ASV) richness, whereas beta diversity analyses demonstrated significant alterations in overall microbial community composition. Postbiotic supplementation increased the relative abundance of *Faecalibacterium*, *Ligilactobacillus*, *Bifidobacterium*, *Prevotella*, *Turicibacter*, and *Fusobacterium*, while reducing *Bacteroides* and *Achromobacter*. Serum IgG concentrations increased, whereas cholesterol and triglyceride concentrations decreased in supplemented dogs ($p < 0.05$). Coat morphology and gloss also improved, without adverse effects on hematological, biochemical, or serum trace mineral parameters. In conclusion, *Bacillus subtilis*-derived postbiotic supplementation improved nutrient utilization, beneficially modulated faecal microbial ecology and fermentation, enhanced selected metabolic and immune responses, and improved coat quality, supporting its potential as a functional dietary strategy for promoting gastrointestinal and systemic health in healthy senior dogs.

Słowa kluczowe

Bacillus subtilis, blood biochemistry, senior dogs, digestibility, gut microbiota, immunity, postbiotic

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