

**P179 - Evaluation of a novel antioxidant formulation for the improvement of canine gastrointestinal health**

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Objective: Reactive oxygen species (ROS) play a significant role in inducing an imbalance of redox homeostasis, leading to oxidative stress, which is associated with various pathological conditions in companion animals, including several inflammation-related gastrointestinal disorders. The potential therapeutic value of nutritional supplementation with antioxidant substances is a subject of ongoing research. The aim of this study was to evaluate *in vitro* through chemical assays the antioxidant properties of three natural substances, namely bromelain (B), quercetin (Q), and powdered shiitake - *Lentinula edodes* - mushroom (LE); and to create a formulation for a feed supplement integrating the three ingredients in the quest to improve gastrointestinal health in dogs in an *in vivo* study.

Methods: In this study, we assessed the total phenolic content (TPC) and antioxidant activity of the three natural substances, B, Q, and LE, as well as a mixture containing these components using a TPC chemical assay, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic diammonium salt acid)) assays respectively, each determination was done in triplicates. The mixture was obtained using the same extract concentration for each individual substance when tested. A novel complementary feed supplement, based on a mixture of the natural substances B, Q, and LE was developed. The safety and efficacy of the complementary feed were evaluated in a 35-day *in vivo* dog study involving 30 dogs randomized into a placebo-controlled (no. 15) and treatment (no. 15) group, and faecal markers of inflammation and immunity were analysed using linear mixed models, where treatment, time, and treatment-by-time interaction were included as fixed-effect predictors, A random intercept was included and pairwise comparisons were made with pairwise t-test. The significance threshold was set at $P < 0.05$

Results: Our findings revealed varying antioxidant activities among the individual natural substances. Remarkably, the formulated combination exhibited the highest ($P < 0.0001$) TPC (4 ± 0.2 mg GAE/g DM (dry matter)) and ABTS (125 ± 3 μ mol TE/g DM) while Q displayed the lowest ($P < 0.0001$) TPC (2 ± 0.2 mg GAE/g DM) and ABTS (11 ± 3 μ mol TE/g DM). Additionally, Q demonstrated the lowest ($P < 0.0001$) DPPH• activity (EC_{50} : 1 ± 10 μ g/mL), with the formulation exhibiting an EC_{50} of 138 ± 10 μ g/mL, LE with an EC_{50} of 231 ± 10 μ g/mL, and Bromelain with an EC_{50} of 434 ± 10 μ g/mL. Analysis of faecal markers of inflammation and immunity revealed a significant reduction in calprotectin, cortisol, histamine, and indole/skatole levels ($p < 0.05$). In contrast, short-chain fatty acid (SCFA) levels increased over time. This reduction suggests a decrease in intestinal inflammation, oxidative stress, and harmful bacterial metabolites, in contrast, the increase in SCFA levels implies an improved gut microbiota composition and enhanced fermentation of dietary fibres.

Conclusions: The study's outcomes have culminated in the creation of an innovative complementary feed supplement, formulated from a blend of the natural constituents, B, Q, and LE. Its efficacy showcased promising outcomes in enhancing canine gastrointestinal well-being. These findings substantiate the viability of this novel natural feed supplement as a dietary modality for ameliorating gastrointestinal health and overall psycho-physical well-being in dogs. Future studies could investigate its long-term effects on a larger and more diverse population of dogs.

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