

Tapping into those 'Gut Feelings': Impact of BL999 (*Bifidobacterium longum*) on anxiety in dogs

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Anxiety is a big concern for many pet owners who want to see their dogs live happy and comfortable lives. Chronic behavior issues can be detrimental to the pet-owner relationship, and in this regard, behavioral issues related to anxiety are among the top reasons that pet dogs are relinquished to shelters. There is mounting evidence in the literature that manipulation of the gut microbiota can influence anxious behavior specifically via the gut-brain axis. Thus, probiotic supplementation has potential as a solution to help alleviate symptoms of anxiety in dogs. We set out to assess the impact of BL999 on anxious behavior in dogs with 24 anxious Labrador retrievers using a holistic approach incorporating both behavioral and physiological parameters. Each dog served as his or her own control and participated in two phases of the study in a crossover design. Dogs were maintained on a complete and balanced diet and supplemented with either BL999 or a placebo for six weeks. All dogs were then subject to a three-week washout period after which they crossed over to the opposite treatment for an additional six weeks. During each phase, the dogs' typical behavior in response to normal day-to-day stimuli was observed directly using a scan sampling method to look for the presence or absence of a defined set of anxious behaviors. In addition, each dog was subject to a formal anxiety test to assess non-social, social and separation anxiety at the end of each supplementation phase. Non-invasive physiological measures were assessed throughout the evaluation. Heart rate and heart rate variability were recorded through the entirety of the formal anxiety test and salivary cortisol concentrations were assessed following the formal anxiety test. We found a significant impact of BL999 on day-to-day anxious behavior (scan samples) with dogs showing an improvement in their behavior when supplemented with BL999 as compared to when they were supplemented with the placebo. This included significant reductions in barking ($P < 0.0001$), jumping ($P < 0.01$), spinning ($P < 0.05$) and pacing ($P < 0.05$). During the formal anxiety test dogs supplemented with BL999 showed increased exploratory behavior in a novel environment compared to when they were supplemented with the placebo ($P < 0.05$). In addition, dogs had reduced salivary cortisol concentrations in response to both exercise and anxiety inducing stimuli when supplemented with BL999 as compared to when they were supplemented with a placebo ($P < 0.001$). When considering cardiac activity, dogs showed a decrease in heart rate ($P < 0.001$) and an increase in heart rate variability (HF: $P < 0.03$; RMSSD: $P < 0.001$) indicating a more positive response to anxiety inducing stimuli when supplemented with BL999 compared to when they were supplemented with a placebo. Thus, from both a behavioral and physiological standpoint BL999 had an anxiolytic effect on anxious dogs and could serve as a useful tool in the development of management plans to improve the well-being of dogs who suffer from anxiety.