CANINE & FELINE BRAIN HEALTH:
GROUNDBREAKING NUTRITIONAL
SCIENCE FROM PURINA
The Purina Institute is strongly committed to advancing nutritional science to benefit pet health. We represent a diverse, global team of more than 500 scientists and pet care experts—including nutritionists, veterinarians, behaviorists, molecular nutritionists, biochemists, microbiologists, and more— with a proven track record of making nutritional discoveries that improve the lives of pets around the globe.
1. FOUNDATIONS OF COGNITIVE SCIENCE IN DOGS AND CATS

Before innovations can be made, science begins with foundational studies to establish and validate testing procedures and determine benchmarks for comparison. In collaboration with our external research partners, Purina has led breakthrough advancements in canine and feline cognition by studying mental processing in the areas of learning, memory and attention through a variety of angles such as feeding schedules and sleep/wake cycles.
This study sought to determine and describe the daily sleep/wake cycle in dogs and to explore the relationship of age, day/night activity, and cognitive function.

**Study Design:** Twenty-four (24) Beagles were separated into three age groups: early adults (1.5-4.5y), late adults (7-9y) and senior (11-14y). They were fitted with collar-mounted activity monitors and housed with 12-hour light/dark cycles. Once acclimated to the light/dark cycles, activity measurements were obtained continuously for 5 consecutive days. Cognitive assessments were performed using three established cognitive tasks (delayed non-matching-to-position task; size discrimination task; and size discrimination reversal task). Cognitive assessment data was compared to the activity data for the late adult and senior dogs.

**Results:** All dogs demonstrated a circadian sleep/wake pattern with high levels of daytime (light cycle) activity compared to nighttime (dark cycle) activity. Total, daytime, and nighttime activity levels changed with age; senior dogs were 51% and 55% less active during the light and dark phase, respectively, compared to early adults. Seniors also became active at least 30 minutes earlier than both younger groups, with activity beginning up to 60 minutes before the lights were turned on for the daytime cycle. Dogs that performed worse (made more errors) on the size task were more active at nighttime. Dogs that performed worse (made more errors) on the size discrimination reversal task had an earlier onset of daytime activity. Interestingly, late adult dogs that performed better (less errors made) on the 90-second delayed non-matching-to-position task were more active during the nighttime. Age-related reduction in, and alterations of, nighttime sleep patterns is likely related to poorer cognitive performance.

**Discussion:** Dogs have a circadian sleep/wake cycle, with higher levels of activity in the daytime than during nighttime. Total, daytime, and nighttime activity levels changed with age; senior dogs are less active compared to early adults. Aging dogs exhibit alterations in nighttime sleep patterns, with more activity at night and earlier onset of daytime activity.

This study was conducted to investigate the influence of age on normal activities in dogs and to evaluate the effect of feeding once daily or twice daily on activity rhythms.

**Study Design:** Forty-eight (48) Beagles of three age groups (early adult [1.5-4.5y], late [7-9y] and senior [11-14y]) were fitted with collar-mounted activity monitors and housed with 12-hour light/dark cycles. They were fed once daily during a 3-week baseline period, then switched to twice daily feeding for 3 weeks. Their rest/activity rhythms during the two periods were compared. Activity measurements were obtained during 3 consecutive 24-hour days at baseline (once-daily feeding), then again for 3 consecutive days beginning at 35 days and 105 days after the dogs had been switched to twice-daily feeding.

**Results:** Dogs are diurnal (3-8 times more activity during day than at night for young and adult dogs) and maintain a fairly consistent circadian sleep/wake rhythm throughout life. As age increased, dogs’ daytime activity decreased; senior dogs’ daytime activity was 42% less than that of early adults and 17% less than that of late adult dogs. Late adult and senior dogs were less active during the nighttime compared to early adult dogs. Dogs fed twice daily increased their nighttime activity by approximately 50% compared to once-daily feeding, but did not alter daytime activity. Dogs fed twice daily also became active earlier in the day compared to once-daily feeding.

**Discussion:** Dogs exhibit strong diurnal rhythms of activity and rest. Aging in dogs is correlated with a shift in activity patterns; they become less active during the daytime and nighttime, which may decrease their caloric intake needs and increase their risk of obesity. However, aging dogs still retain diurnal activity rhythms, with daytime activity greater than nighttime activity. Senior dogs are less active during the day than their younger counterparts. Feeding adult dogs twice daily, as opposed to once daily, results in an increase in nighttime activity and an earlier start to their day; this effect was less pronounced in seniors. Regardless of age or feeding frequency, nighttime activities comprised approximately one-fourth of their total activity level.

This study in dogs was designed to assess the impact of aging on sleep/wake activity and the impact relative to the frequency of feeding. (Note: behavioral sleep refers to sleep measured through the dog’s behavior and activity, not measured through brain activity.)

**Study Design:** Forty-eight (48) Beagles of three age groups (early adult [1.5-4.5y], late [7-9y] and senior [11-14y]) were fitted with collar-mounted activity monitors and housed with 12-hour light/dark cycles. Activity measurements were obtained 3 consecutive 24-hour days at baseline (once-daily feeding for 3 weeks), then again for 3 consecutive days beginning at 35 days and 105 days after the dogs had been switched to twice-daily feeding to determine the dogs’ sleep time and sleep efficiency, nap frequency, how often dogs awoke during the night, and what time dogs became active in the morning.

**Results:**

**Effect of age:** Young adult dogs were awake 90 minutes more each 24-hour cycle than middle-aged and senior dogs, with 40-53 minutes of that additional time occurring during the daytime hours.

**Effect of feeding frequency:** When the dogs were fed twice daily, they were awake for a total of just over an hour (63 minutes) more per day. They also napped less frequently, but for about 1 minute longer during each nap, resulting in about the same amount of total time spent napping per day. Twice-daily feedings reduced sleep efficiency (the time actually slept compared to the total time laying down to sleep) by up to 8%, and dogs fed twice daily were awake and active 20-30 minutes earlier in the day.

**Discussion:** Feeding dogs, including seniors, twice daily (as opposed to once daily) increases their total time awake during the day and leads to a 20-30 minute earlier start to their day’s activity.

This study evaluated dogs with mild to moderate cognitive impairment to assess if cognitive impairment would be related to alterations in diurnal behavioral and physiological rhythms.

**Study Design:** Based on several previously validated cognitive tests to assess working memory performance and selective attention capacity, 33 Beagles aged 9-16 years were identified to have cognitive impairment despite a lack of typical signs of cognitive dysfunction syndrome. The dogs were then fitted with activity monitor collars, fed twice daily and housed with controlled 12h light/12h dark cycles. Dogs were switched from once-daily feeding to twice-daily feeding on Day 1 of the study; cognitive testing began 10 days later and involved a training period from Day 10 to Day 20 on a task to assess baseline memory performance and dogs were then sorted into low memory performer and moderate memory performer groups. The two groups were further divided into two subsets, and one subset (randomly selected) was trained for 30 days on a task to assess learning and selective attention. Several previously validated cognitive tests were performed to assess working memory performance and selective attention. Activity measurements were obtained over a period of 5 consecutive 24-hour days from Day 1 through Day 4 of the study. The dog’s sleep/wake activities were recorded. Core body temperature was measured.

**Results:** Dogs over 11 years of age had significantly (29%) lower daytime activity and spent more time napping during the day, but the total daytime activity was not related to cognitive performance. Better performance on the 90-second delay task that assessed working memory performance correlated with fewer daytime naps, suggesting that increased daytime napping may partially predict a loss of working memory ability but does not necessarily predict a more severe cognitive decline because performance on other cognitive tasks did not indicate impairment in other aspects. Lower daytime core body temperature peak values and/or increased daytime napping, but not nighttime activity, were more significantly associated with decreased cognitive performance. And because most of the dogs in this study were found to be cognitively impaired on testing but did not display many of the typical signs of cognitive dysfunction, this supports the hypothesis that cognitive impairment may be detected earlier with testing, before clinical signs are observed.

**Discussion:** Dogs over 11 years of age are less active and nap more during the day. Increased napping may be an early indicator of some memory loss. Lower afternoon peak core body temperature may be a biomarker that predicts cognitive impairment, but it remains to be seen if this is clinically relevant. Cognitive testing may detect cognitive impairment prior to the appearance of clinical signs.

This study sought to further establish the relationship between various cognitive domains (visuospatial memory, object discrimination learning, and selective attention).

**Study Design:** Thirty-six (36) Beagles ranging from 9-16 years of age were trained on a spatial memory task (vDNMP) to establish a baseline level of working memory function and sorted into three groups based on baseline testing: high memory performers, moderate memory performers, and low memory performers. They were then trained over 30 days on a variable object oddity task to assess learning and selective attention. They were re-tested on spatial memory 45 days after the baseline to confirm the robustness of working memory function, followed by a new test to measure psychomotor function.

**Results:** Dogs over 12 years of age performed worse on the variable object oddity task compared to dogs 9-12 years of age regardless of their overall cognitive performance. Performance on the spatial memory test did not predict performance for some aspects of learning and attention processes; the tasks assess separate and at least partially independent functions. Dogs that performed well on the spatial memory task at baseline still performed well on the task when re-tested 45 days later. Older dogs (12+) performed better on the two-choice discrimination (attention) task compared to dogs 9-12 years of age despite no significant differences in performance of the spatial memory test.

**Discussion:** The areas of the brain that account for working memory appear to be at least partially independent from those responsible for learning and attention. If one type of learning or memory is affected, it doesn’t necessarily mean that other functions are affected. It is possible to improve types of learning in older dogs as well – you CAN teach an old dog new tricks!

2. ANTIOXIDANTS, OMEGA-3 FATTY ACIDS AND BRAIN PROTECTION

Metabolic changes and risk factors associated with brain aging and age-related cognitive decline can be targeted with nutritional interventions that support healthier brain aging.
This study sought to determine the effect of long-chain polyunsaturated fatty acid (LCPUFA) supplementation on cognitive function of puppies.

**Study Design:** Five (5) litters of mixed-breed puppies (40 puppies total) were divided into two groups: one-half of each litter (20 puppies total) was fed a placebo diet, and the other half was fed a diet supplemented with LCPUFA from 3 to 16 weeks of age. Each puppy was assessed for mental stability, problem solving (U-maze and long maze test), memory (long maze test) and cue association (T maze) using validated tests between 8 and 16 weeks of age.

**Results:** No significant differences were found between the mental stability of the placebo or treatment group or in performance in the U-maze, although LCPUFA-supplemented puppies showed a numerical trend of improved performance. No significant difference was found between groups in the first trial in the long maze; on the second trial in the long maze, LCPUFA-supplemented puppies completed the maze significantly faster than the control puppies with a nonsignificant but reduced median and minimum number of errors.

**Discussion:** Significantly improved time of completion of the long maze in the LCPUFA-supplemented puppies compared to the placebo-fed puppies suggests that LCPUFA supplementation enhanced memory, and may enhance trainability.

This study tested a hypothesis that nutritional modifications targeting metabolic pathways and risk factors associated with normal brain aging would support healthy brain aging. The researchers tested the long-term effectiveness of a proprietary blend of nutrients (comprised of fish oil, arginine, B vitamins and antioxidants) on the cognitive function of middle-aged and old cats.

**Study Design:** Thirty-two (32) cats, ranging from 5.5 to 8.5 years in age, were divided into two groups: a control group (no nutrient blend) and a treatment group (with nutrient blend) with equivalent age, body weight and cognitive performance at the start of the study. Body weight was monitored and recorded at 2-week intervals. Blood samples were obtained prior to the start of the study and again at 200 and 345 days (end of study) and comparisons were made between the 2 groups for levels of folic acid, B₁₂, homocysteine, total antioxidant status and erythrocyte fatty acid profiles.

**Results:** The test group (with nutrient blend) performed better on cognitive tests and, in most cases, the difference was statistically significant. [Performances on the egocentric (spatial learning relevant to own body position), size discrimination (visual learning) and reversal (flexibility and executive function) tasks, and DNMP (delayed non-matching to position task, which assesses spatial working memory and demonstrates learning and interacting with the environment) task were statistically significant. Test group cats responded better on the landmark test, but the results were not significant]. The test group cats were more accurate and completed more reversal learning problems than the control group cats. The test group cats showed signs of performance improvement within 30 days of starting the diet and performed better on the DNMP test at the end of the year compared to baseline, whereas the control group cats did not. The test group cats had significantly higher folic acid levels compared to the control group, but no other statistically significant differences were found in blood analyte levels tested at the end of the study. Body weight was not significantly different between the groups at the conclusion of the study. Consumption of the test diet resulted in cognitive benefits, either by enhancing cognition, through neuroprotective effects, or possibly both.

**Discussion:** The combination of fish oil, B vitamins, antioxidants, and arginine has significant cognitive performance benefits and may slow age-related decline in cognitive function in normal middle-aged and older cats.

This study was performed to determine if a nutrient blend of fish oil, B vitamins, antioxidants and arginine confers cognitive benefits to aged dogs.

**Study Design:** Aged dogs (9.1 to 11.5 years of age) were fed either a control diet (complete and balanced, but not supplemented) or the test diet with the blend of fish oil, B vitamins, antioxidants (including Vitamin C, Vitamin E and selenium) and arginine. Cognitive testing was performed prior to the study to establish a baseline, and throughout the 6-month trial: landmark discrimination (locating objects in space based on external cues) and egocentric discrimination (spatial learning relevant to own body position) with reversal (to measure flexibility and executive function) were performed and compared to baseline and between groups. Metabolomics analysis was performed and compared between groups.

**Results:** Although there was no significant difference between the control and supplemented (test diet) dogs on the basic landmark and egocentric tasks, the supplemented dogs performed significantly better than controls on the more complex landmark and egocentric reversal tasks. Supplemented dogs had higher plasma levels of arginine, alpha-tocopherol (Vitamin E), and the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

**Discussion:** Long-term supplementation with a nutrient blend of fish oil, B vitamins, antioxidants and arginine provides cognitive benefits, particularly with more complex cognitive tasks – such as those that assess executive function, which includes goal-oriented behavior, decision making, problem solving, planning, organizing and sequencing tasks.

Metabolism of glucose, the primary energy source for the brain, becomes less efficient with aging. The resulting energy depletion of neurons can lead to memory loss, slower learning, and reduced attention. The neurons of dogs with epilepsy are also less able to utilize glucose as an energy source, resulting in similar oxygen starvation and altered function for some areas of the brain. Providing the neurons with an alternative energy source – such as ketone bodies, derived from the breakdown of medium-chain triglycerides (MCTs) – can help mitigate the glucose hypometabolism. Metabolites of MCTs may be of benefit to dogs with epilepsy through alternative mechanisms.
This study was intended to investigate the hypothesis that dietary supplementation with medium-chain triglyceride oil (MCT oil; also called medium-chain triacylglycerols, or TAG) would improve cognitive function in senior dogs.

**Study Design:** Twenty-four (24) Beagles 7.5-11.6 years of age were assessed at baseline through cognitive testing and then fed either a control diet (no MCT oil) or a diet containing 5.5% MCT oil for 8 months. Cognitive testing (landmark, egocentric, and variable object oddity protocol tasks) was performed beginning one week after initiation of the diets and continued through the study; dogs were tested daily on one task at a time. A complete blood count (CBC), serum chemistry profile, and serum β-hydroxybutyrate (BHB) levels were measured prior to the start of the study and again at 4 and 8 months.

**Results:**

**Cognitive testing:** The MCT oil group had fewer errors than the control group on landmark testing, and the results were significant for the more difficult of the landmark testing tasks. The MCT oil group also scored higher in egocentric task tests, with significant effects in the more advanced versions of the tests, and also performed better than the control group on the variable object oddity test when there were two or three distractors in the tasks.

**Laboratory testing:** Dogs in the MCT oil diet group had significantly higher levels of BHB, indicating that the MCT oil diet increased ketone bodies in the bloodstream. All CBC and chemistry values were within normal limits throughout the course of the study.

**Discussion:** MCT oil supplementation can improve cognitive performance in dogs with age-related cognitive decline, including promoting memory, attention and trainability in as little as 30 days.

This study evaluated the effects of a diet containing a proprietary blend of medium-chain triglycerides, omega-3 fatty acids, B vitamins, and antioxidants on the clinical signs observed in dogs with cognitive dysfunction syndrome (CDS).

**Study Design:** Pet dogs were assessed based on owner’s responses on a behavior and health (DISHAA) questionnaire assessing six categories associated with CDS: disorientation, altered social interaction, sleep-wake cycle disturbance, loss of house training, anxiety, and altered activity. (Collectively, these categories are referred to as DISHAA.) Eighty-seven (87) pet dogs were randomly assigned to three groups: control (nutritionally complete and balanced diet with 0% MCT oil-blend), 6.5% MCT oil-blend diet, and 9% MCT oil-blend diet. The dogs were assessed on the six DISHAA categories prior to the trial (baseline) and at 30 and 90 days after beginning the trial.

**Results:** Dogs fed the 6.5% MCT oil-blend diet exhibited significant improvements in five out of six DISHAA categories (all but sleep-wake cycle disturbance) at the 30-day assessment, and significant improvements in all six of the DISHAA categories at the 90-day assessment, as compared to baseline assessment. Dogs on the 9% MCT oil-blend diet had significant improvements in DISHAA categories, but less so than dogs consuming the 6.5% MCT oil-blend diet: this may have been related to potentially reduced intake due to diet acceptance palatability issues.

**Discussion:** Feeding a proprietary blend of MCT oil, omega-3 fatty acids, B vitamins, antioxidants and arginine results in improvement in the clinical signs of cognitive dysfunction syndrome in dogs. Signs of improvement may be observed within the first month of feeding.

This study was performed to determine if a diet containing 5.5% of a proprietary blend of medium-chain triglyceride (MCT) oil, which increases ketone body formation, would affect seizure activity in dogs with idiopathic epilepsy.

**Study Design:** 6-month prospective, randomized, double-blinded, placebo-controlled, crossover dietary trial comparing a placebo (control) diet and a diet containing 5.5% of a proprietary blend of medium-chain triglyceride (MCT) oil. Twenty-one (21) dogs that were 6 months to 12 years of age with idiopathic epilepsy who were being treated chronically with at least one antiepileptic medication and had at least 3 seizures in the past 3 months were fed either the placebo diet or the MCT diet for 3 months, then crossed over to the opposite diet for 3 months. Seizure frequency, clinical and laboratory data were collected. No changes were made to the dog’s anti-epileptic medications during the trial.

**Results:**

**Seizure frequency:** Feeding the test diet containing MCT oil significantly reduced seizure frequency (the number of seizures per month). 15 dogs (71%) showed an overall reduction in seizure frequency in response to the MCT-containing diet, with three dogs (14%) achieving complete freedom from seizures and an additional seven dogs showing a 50% or greater reduction in seizure frequency. Six dogs (29%) showed no response to the MCT oil-containing diet.

**Seizure day frequency:** Feeding the test diet containing MCT oil significantly reduced seizure day frequency (the number of days in a month with seizure occurrence). Three dogs (14%) achieved complete freedom from seizures; seven dogs (33%) had over 50% reduction in seizure day frequency; seventeen dogs (81%) had an overall reduction in seizure day frequency; and four dogs (19%) showed no response to the MCT diet.

**Rapidity of MCT effect:** The effect of the MCT diet was as early as day one for some dogs and persisted throughout the diet period.

**Effect on drug levels:** The MCT diet did not cause significant changes in the plasma concentrations of phenobarbital or potassium bromide.

**Discussion:** A diet with 5.5% of a proprietary blend of medium-chain triglyceride oil significantly reduced the seizure frequency and seizure day frequency of dogs with idiopathic epilepsy. This may serve as a valuable adjunct approach to traditional veterinary therapy in the management of dogs with this chronic neurological condition.


This study sought to determine if dogs with idiopathic epilepsy exhibit ADHD-like behaviors, similar to humans, and whether a ketogenic diet could affect those behaviors.

**Study Design:** 6-month prospective, randomized, double-blinded, placebo-controlled, crossover dietary trial comparison of a placebo (control) diet and a diet containing 5.5% medium-chain triglyceride oil (MCT oil). The dog owners, investigators, and statisticians were blinded throughout the study. Twenty-one (21) dogs that were 6 months to 12 years of age with idiopathic epilepsy who were being treated chronically with at least one antiepileptic medication and had at least 3 seizures in the past 3 months were fed either a placebo diet or the MCT oil diet for 3 months, then switched to the opposite diet for 3 months (each dog was its own control for comparison). Owners, who were blinded to the food their dog was receiving, completed a validated behavioral questionnaire at the end of each three-month period to report on their dog’s behavior during that interval. No changes were made to antiepileptic medications during the trial.

**Results:**

**Behaviors:** During the placebo period, excitability (with more than half of the owners reporting “extreme excitability”) and chasing (of squirrels, cats, birds and other small animals) were noted in the dogs. Two-thirds of owners also reported that their dogs were always easily distracted and were less likely to obey a sit or stay command.

During the MCT oil diet period, excitability and chasing were reported by owners; chasing behaviors, but not excitability, were significantly reduced compared to the placebo diet. Dogs on the MCT oil diet also exhibited a significant reduction in stranger-directed fear. Trainability was improved in dogs consuming the MCT oil diet, but was not significantly improved compared to the placebo.

**Seizure activity:** Median seizure frequency per month and median seizure days per month were significantly lower on the MCT diet compared to the placebo diet.

**Discussion:** Dogs with idiopathic epilepsy can also exhibit ADHD-like behaviors – predominantly high excitability and chasing behavior, resulting in low trainability scores. Feeding a diet with 5.5% MCT oil reduced chasing behavior as well as stranger-related fear.

This study was performed concurrently with the clinical trial evaluating the effectiveness of a ketogenic medium-chain triglyceride (MCT) diet for dogs with refractory idiopathic epilepsy (Law et al, 2015).

**Study Design:** 6-month prospective, randomized, double-blinded, placebo-controlled, crossover dietary trial comparing a diet containing 5.5% of a proprietary blend of medium-chain triglyceride (MCT) oil and a control diet containing no MCT but an equivalent amount of long-chain triglycerides. Twenty-one (21) dogs that were 6 months to 12 years of age with idiopathic epilepsy who were being treated chronically with at least one antiepileptic medication and had at least 3 seizures in the past 3 months were fed either the placebo diet or the MCT diet for 3 months, then crossed over to the opposite diet for 3 months. No changes were made to the dog’s anti-epileptic medications during the trial. Blood samples were obtained at the end of each 3-month diet phase (study days 90 and 180) and analyzed using chromatography to determine the metabolic response to the MCT diet.

**Results:** Consumption of the MCT diet resulted in significant changes to the fasting serum metabolic profiles compared to the placebo diet. Sixteen metabolites were significantly altered by the MCT diet, all of which were categorized as phosphatidylcholine and acylcarnitine metabolites. Phosphatidylcholine metabolites were more significantly affected by the MCT diet.

**Discussion:** Consumption of the MCT diet resulted in a global shift in lipid metabolism. In particular, the increased levels of C17:0 fatty acids may contribute to the clinical effects of the MCT diet: the C7:0 metabolites of these fatty acids are proposed to have neuroprotective and anticonvulsant properties.

4. THE GUT-BRAIN AXIS, PROBIOTICS AND ANXIETY

The role of the intestinal tract goes far beyond digestion. It is in constant communication with the brain, through the gut microbiota. Research has shown links between dysbiosis (an imbalanced or maladapted microbiota) and health conditions such as cognitive changes and canine anxiety. Altering the gut microbiota via nutritional interventions has the potential to facilitate cross-talk between the gut and brain, and influence behavior and mood.
This study evaluated the effects of a specific strain (BL999) of *Bifidobacterium longum* on anxious behaviors in dogs.

**Study Design:** 15-week blinded, placebo-controlled crossover study with 24 anxious Labrador Retrievers. All dogs were maintained on a complete and balanced diet supplemented with either a placebo or the probiotic BL999 for 6 weeks. Following a three-week washout period, each dog was placed on the opposite supplement (placebo or BL999) of the previous period. During each phase, the dogs’ behavior in response to normal day-to-day stimuli was observed for a defined set of anxious behaviors. Formal anxiety evaluations were also performed to assess non-social, social and separation anxiety behaviors at the end of each supplementation phase. Heart rate and heart rate variability were recorded through the entirety of the formal anxiety test and salivary cortisol levels were assessed prior to and following the formal anxiety test.

**Results:** Feeding BL999 resulted in a significant reduction in anxious behaviors such as barking, jumping, spinning and pacing. During the formal anxiety test, BL999-supplemented dogs showed increased exploratory behavior in a novel environment compared to their behavior on the placebo.

Additionally, 83% of dogs studied had lower salivary cortisol levels in response to both exercise and anxiety-inducing stimuli when supplemented with BL999. After 6 weeks of supplementation with BL999, dogs showed a decrease in heart rate and an increase in heart rate variability, indicating a more positive response to anxiety-inducing stimuli.

**Discussion:** Anxious dogs treated with BL999 were less reactive, more calm and potentially in a better affective state when experiencing anxiety-provoking stimuli. In addition, BL999-supplemented dogs exhibited less daily anxious behavior than when supplemented with a placebo. BL999 has 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.