‘Oiling the Brain’ or ‘Cultivating the Gut’: Impact of Diet on Anxious Behavior in Dogs

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Abstract

Nutritional solutions to anxiety in dogs should be considered as part of a management plan for improving the well-being of anxious dogs and preserving the pet-owner relationship. A broad overview of the impact of diet on behavior in dogs is presented with specific recommendations for two potential interventions that show promise in alleviating signs of anxiety in dogs. A holistic approach incorporating both behavioral and physiological measures demonstrates a positive impact of both fish oil and the probiotic *Bifidobacterium longum* on mediating anxious behavior and stress responses in anxiety-prone dogs.

Scope of the Problem – How Prevalent Is Anxiety in Dogs?

Anxiety is a big concern for pet owners who have developed close bonds with their dogs and want to see them live happy and comfortable lives. Inevitably, living with an anxious dog can be as emotionally draining for the owner as for the dog itself. It has been estimated that 29% of pet dogs exhibit signs of anxiety[1,2]; however, this may be a gross underestimate as many owners accept anxious behavior as “just the way they are,” and thus do not seek formal diagnosis for their pets. Anxiety-related issues include separation anxiety, noise phobias (e.g., thunderstorms or fireworks), fear of strangers, fear of novelty, and fear-related aggression. Up to 70% of behavioral disorders occurring in dogs can be attributed to some form of anxiety.[3]

The term “anxious” is used to describe dogs that are hypervigilant and/or hyperreactive, seemingly anticipating a negative outcome and experiencing negative emotional arousal even before a situation he or she perceives as inevitable or dangerous occurs.[4,5] An anxious dog has an increased probability of reacting negatively to any change in environment, be it internal or external.[3] Some dogs have a genetic predisposition toward an anxious temperament. For others, their anxiety may develop as a result of early environment or experiences. Signs of general anxiety in dogs include hypervigilance/wandering around unable to settle, reluctance to take treats, excessive barking/attention seeking, panting when not hot, repeated yawning, lip licking/smacking and or body shaking, house soiling, compulsive licking, and/or aggression.[6,7] Animals have highly evolved stress mechanisms enabling them to deal with acute stressors in an efficient manner by eliciting the appropriate behavioral and physiological responses needed to help them escape a threat. These stress responses become problematic when an individual is hypervigilant to stressful stimuli and/or perceives threats when none exist, thus remaining in a state of constant stress from which they are unable to escape.

Behavioral issues related to anxiety are among the top reasons that pet dogs are relinquished to shelters.[8] Chronic behavioral issues with a pet may be detrimental to the relationship between the owner and that pet, damaging the bond that connects them. This loss of connection may serve as an important determinant in the ultimate decision to relinquish a pet. Many dogs with histories of behavioral issues are deemed unsuitable for rehoming and/or are returned after adoption leaving them on a likely path toward euthanasia.[9-12] In addition to the obvious well-being concerns for dogs that suffer from anxiety, there is evidence to suggest that highly anxious pets may actually have a shorter life span than more easygoing pets. If anxious dogs are stressed by many day-to-day stimuli, they may be living in a state of chronic physiological stress.[13] In other words, a pet with a thunderstorm phobia living in a storm-prone area or a pet with separation anxiety that is left home alone each day may suffer from repeated stressful episodes over a lifetime. Prolonged stress is related to a number of variations in hormone and immune regulation and has been implemented in the development of various disease states and shortened life spans in several

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species.[13] Given the impact that anxiety can have on their dog's physical and emotional well-being, many pet owners are looking for ways to help their dogs cope with day-to-day stressors and allow them to maintain more positive emotional states.

State of the Art – Impact of Diet on Behavior in Dogs

For many dogs, anxious behavior can be mediated though behavior modification and in extreme cases through pharmaceutical intervention. Pharmaceutical interventions may play an important role in helping anxious dogs succeed during behavior modification by aiding them to reach an optimal mental state in which they can better respond to behavior modification. However, pharmaceuticals can be expensive, and some owners are hesitant to use these medications because of the potential for side effects or negative perceptions around "drugging" their pets. Many pet owners would prefer to have a more "natural" aid to help with their anxious dog. Nutrition is often overlooked as a contributing factor to help address behavioral problems in dogs. However, altering diet to manipulate the availability of precursors for the hormones and neurotransmitters that regulate behavior has merit as a means to mitigate many behavioral issues.[14]

A small number of studies in the literature support the positive impact of diet on problematic behavior in dogs. These include supplementation with milk[3,15] or fish[16] derivatives, manipulation of amino acid or protein levels,[17-20] a combination of the above,[21] and homeopathic[22] or herbal supplementation.[23]

Two studies have investigated the impact of bovine milk derivatives on behavior in dogs. Beata and colleagues[3] compared the behavior of anxious dogs supplemented with alpha-casozepine with the behavior of dogs supplemented with selegiline, a known anxiolytic. When comparing general anxious behavior of dogs through use of the emotional disorders evaluation in dogs (EDED) scale, 9/19 dogs (47%) treated with selegiline and 10/19 dogs (53%) treated with alpha-casozepine showed marked improvement.[3] Unfortunately, the study design did not include a placebo treatment (i.e., group fed a control diet) or control for the effect of the behavior modification plans that the dogs were undergoing at the same time they were on treatment (i.e., group with behavior modification alone). Thus, it is difficult to determine the magnitude of the effect of these supplements on anxious behavior. In a later study, a diet formulated with caseinate hydrolysate fed to anxious dogs for 65 days was found to reduce reactivity, some anxious behavior and cortisol levels but not heart rate.[15] Taken together, this work provides evidence to warrant further investigation into the potential of these bovine milk derivatives as functional ingredients for mitigating stress in dogs.

Recently, Landsberg and colleagues[16] examined the impact of a fish-protein derivative on fearful behavior in thunder-phobic dogs. The provision of fish hydrolysate reduced fearful behavior and decreased blood cortisol levels in response to simulated thunder.[16] Dogs were considered "cortisol responders" to thunder if their cortisol levels were at least double those collected during baseline. Following treatment, 2/9 control dogs (22%), 7/10 dogs (70%) provided a low dose (70%), and 5/10 dogs provided a high dose (50%) showed a decrease in their cortisol response to the sound of thunder so they were no longer considered to be responders.[16] The impact on cortisol response was most prominent toward the end of the study, hence the authors suggest that long-term treatment may increase the anxiolytic effectiveness of fish hydrolysate.

A handful of studies have investigated the impact of amino acids and protein levels on the behavior of dogs. Araujo and colleagues[20] explored the impact of L-theanine (an amino acid found in green tea) supplementation on five anxious dogs with a fear of unfamiliar people. Dogs treated with L-theanine were more willing to approach and interact with an unfamiliar person than dogs in a control group.[20] Bosch and colleagues[19] investigated the impact of tryptophan supplementation on mildly anxious dogs. The behavior of dogs fed a diet supplemented with tryptophan were compared with control dogs through owner observation and a formal anxiety test. Despite marked increases in plasma tryptophan concentrations (37% increase), there were no detectible changes in behavior that could be attributed to a dietary effect when considering either the owner reported data or results from the formal anxiety test. There was also no measurable impacts on salivary cortisol levels even after tryptophan supplementation for eight weeks.[19] As part of the same study the authors also investigated the effect of a diet formulated with a combination of tryptophan, beet pulp, salmon oil, soy lecithin, and green tea extract, and found no impact on anxious behavior.[19]

DeNapoli and colleagues[18] examined the impact of tryptophan supplementation coupled with dietary protein level on dogs with dominance aggression, territorial aggression or hyperactivity issues. Owners were asked to score their dog's behavior on a daily basis throughout the treatment. No significant changes in behavior were detected within any of the three dog behavior types for any of the dietary treatments.[18] When the data were pooled together and the analysis was run across groups, some dietary effects emerged. Increased tryptophan led to a reduction in aggression scores for some dogs under specific circumstances, but there were no changes in scores for hyperactivity. The authors suggest that this effect was achieved either through supplementation with tryptophan directly or by manipulating protein levels to increase the ratio of tryptophan to large neutral amino

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acids in the blood. However, no significant differences in plasma tryptophan or serotonin were detected among dogs on the different diets.[18] In an earlier study, Dodman and colleagues[17] examined the impact of dietary protein level on dominance aggression, territorial aggression or hyperactivity in dogs. Protein content was manipulated within test diets, and owners were asked to assess changes in their dog's behavior. No detectible changes in behavior were observed for controls, hyperactive or dominant aggressive dogs. For a subset of seven fearful dogs that were territorial aggressive, feeding a low-to-medium protein diet led to some reduction in their aggressive tendencies. The authors concluded that while a reduction of dietary protein may help a subset of dogs, it is not generally useful in the treatment of behavior problems in dogs.[17] Based on the evidence at hand it is unclear whether the level of protein or the specific makeup of the protein provided would have a meaningful influence on behavior. Further investigation is needed to draw any conclusions around the impact of dietary protein or amino acids on problem behavior in dogs.

One study to date has explored a combination effect utilizing a diet containing both alpha-casozepine and added tryptophan. Small dogs (<15 kg) fed a diet containing tryptophan and alpha-casozepine showed a reduced urine cortisol-to-creatinine ratio after being taken into a veterinary clinic to have their nails trimmed as compared to an earlier nail trim when being fed a control diet.[21] The authors state that it is unclear whether this effect was due to the alpha-casozepine, increased protein in the diet, or a combination of the two factors.

The final studies of note investigated the impact of homeopathic or herbal blends on behavior in dogs. Cracknell and Mills[22] tested a homeopathic blend comprised of phosphorus, rhododendron, borax, theridion, and chamomilla with dogs that were reactive to firework noises. Owners were tasked with dosing their dogs, and behavioral changes were noted through owner observation. Equal improvement in behavior was noted between supplementation with the homeopathic remedy and a placebo, with 26/40 owners (65%) in the placebo group and 25/35 owners (71%) in the treatment group reporting improvements in behavior. The authors caution that this is a good example of the impact the "placebo effect" can have on perceptions of improvement, thus the results of uncontrolled tests regarding the impact of supplements on behavior should be interpreted with caution. In a later study, a proprietary blend of extracts from *Magnolia officinalis* and *Phellodendron amurense* showed some efficacy of reducing the magnitude of fearful behavior of dogs in response to thunder.[23] Anxious behavior in response to thunder improved for 12/20 dogs (60%) as compared to a placebo treatment in which 5/20 dogs (25%) showed improvement.[23] The later study shows some promise toward an herbal remedy helping to alleviate symptoms of anxiety for some dogs.

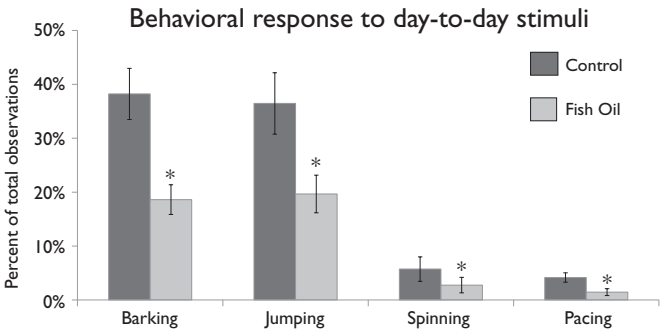
'Oiling the Brain' – Fish Oil as a Potential Solution to Anxiety in Dogs

Ample evidence in the literature exists to support the use of fish oil as a potential solution to alleviate symptoms of anxiety, depression and hyperactivity in numerous species.[24-27] Although the precise mechanism by which fish oil influences behavior is uncertain, omega-3 fatty acids have anti-inflammatory and antioxidative properties and play a role in neuroplasticity as well as modulation of neurotransmitters, all of which could lead to psychotropic effects.[27] At least for humans, consumption of higher levels of omega-3 fatty acids on a daily basis results in a larger volume of gray matter in the areas of the brain related to mood regulation.[28] In turn, low dietary intake of omega-3 fatty acids has been linked to mood disorders, social anxiety disorders, hostility, and impulsive behavior.[28,29,30] Omega-3 fatty acids are of vital importance within cell membranes and in connections between nerves,[31] and low levels of omega-3 fatty acids in the brain may result in continual signal disruption, which, in turn, could result in behavioral issues. Thus, the consumption of omega-3s may actually provide a protective effect against mood-related problems.[28]

Fish oil has been suggested as a supplement to help children with attention-deficit hyperactivity and anxiety disorders,[32] and a recent study found that supplementation with fish oil lowered anxiety and increased cognitive performance in nonhuman primates.[33] In addition, daily supplementation with fish oil has been shown to reduce anxiety symptoms in people who had not previously been diagnosed with an anxiety disorder[34] and to reduce anxiety and anger scores in substance abusers.[35] Omega-3 fatty acids have also been shown to have an antidepressant effect on rats subject to the forced swim (anxiety inducing) test.[36,37] Last, omega-3 fatty acids influence the same pathways associated with antianxiety medications[14,27] and have been shown to have similar effects as Fluoxetine,[38] a commonly prescribed treatment for anxiety in dogs.

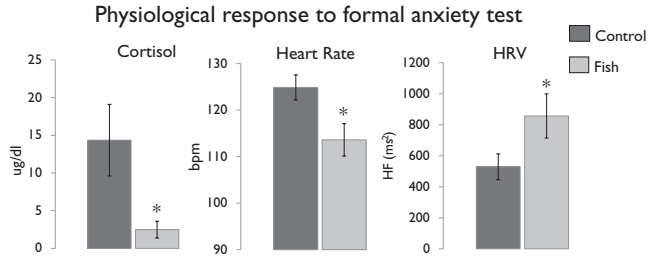
To assess the impact of fish oil on anxious behavior in dogs, Nestlé Purina tested 24 anxious Labrador Retrievers using a holistic approach incorporating both behavioral and physiological parameters. Each dog served as his or her own control and participated in two phases of the study. Dogs were maintained on a complete and balanced control diet for eight weeks and then transitioned to a complete and balanced diet with increased levels of fish oil for 12 weeks. During each phase the dogs' typical behavior in response to normal day-to-day stimuli was observed directly each week using a scan sampling method to look for the presence or absence of a defined set of anxious behaviors (e.g., spinning, pacing, jumping, barking). In addition, each dog was subject to a formal behavioral evaluation to assess nonsocial, social and separation anxiety at the end of each feeding phase. Noninvasive physiological measures were assessed through-

Figure 1. Behavioral change in response to day-to-day stimuli. Dogs exhibited less anxious behavior when fed a complete and balanced diet with added fish oil compared to a complete and balanced control diet.



* indicates a significant difference P<0.05.

Figure 2. Physiological changes in response to formal anxiety test. Dogs were less reactive (lower salivary cortisol and lower heart rate) and in a more positive affective state (higher heart rate variability (HRV)) during a formal anxiety test when fed a complete and balanced diet with added fish oil compared to a complete and balanced control diet.



* indicates a significant difference P<0.05.

out the evaluation. Heart rate and heart rate variability were recorded through the entire formal anxiety test and salivary cortisol concentrations were assessed following the formal anxiety test.

A significant impact of the fish oil diet on day-to-day anxious behavior (scan samples) was found with 21/24 dogs (87%) showing an improvement in their behavior when fed the fish oil diet as compared to when they were fed the control diet (Figure 1). This included significant reductions in barking, jumping, spinning, and pacing. In addition, 21/24 dogs (87%) had reduced salivary cortisol concentrations in response to the formal anxiety test when fed the fish oil diet than when fed the control diet. When considering cardiac activity, 17/24 dogs (70%) showed a decrease in heart rate and an increase in heart rate variability, indicating a more positive response to the formal anxiety test when fed the fish oil diet compared to when they were fed the control diet (Figure 2). Thus, from both a behavioral and physiological standpoint increased intake of fish oil had a calming effect on anxious dogs.

'Cultivating the Gut' – Impact of *Bifidobacterium longum* on Anxiety in Dogs

It is generally recognized that the gut and the brain are engaged in constant bidirectional communication through what has been coined the "gut-brain axis." The precise mechanism by which this occurs can only be speculated; however, there are several plausible hypotheses. There is evidence to suggest that gut microflora present during development may directly influence receptor expression in the brain.[39] In addition, gut microflora produce neurotransmitters that alter brain chemistry (e.g., GABA,[40] BDNF[41]). There also is evidence to suggest that gut microflora may program brain development and stress response.[42] All in all, gut-brain communication may involve neural pathways as well as immune and endocrine mechanisms.[43] Regardless of the specific mechanism, there is now robust evidence that gut microbiota can signal the central nervous system (brain) via vagal pathways within the enteric nervous system (gut).

There is mounting evidence in the literature that manipulation of the gut microbiota can influence anxious behavior specifically. There are studies that demonstrate that anxious behavior transfers with gut microbes,[44] even in the absence of the nonbehavioral phenotype associated with anxiety (e.g., obesity),[45] or that anxious states can be influenced by the absence of gut microbiota.[42,46,47] Of particular interest is the anxiolytic properties of specific probiotics. For example, oral administration of some bacteria (e.g., *Camphylobacter jejuni*) has an anxiety-provoking effect in mice,[48] while oral administration of others (e.g., *Lactobacillus rhamnosus*) reduces anxiety in mice.[39] A study investigating the response of both rats and humans[49] to a probiotic blend containing *Bifidobacterium longum* reduced anxiety-like behavior in rats and alleviated psychological distress in human volunteers. There is evidence to suggest that at least for *B. longum*, this anxiolytic effect works through the vagal pathway.[50]

To assess the impact of a proprietary strain of *B. longum* on anxious behavior in dogs, Nestlé Purina assessed 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 a crossover design. In the first phase of the study, dogs were maintained on a complete and balanced diet for six weeks with 12 dogs being supplemented with a proprietary strain of *B. longum* and the other 12 dogs being supplemented with a placebo. Following this phase, there was a three-week washout period after which the dogs were reversed with the dogs receiving the opposite treatment as during the first phase of the study. During each phase, the dogs' typical behavior in response to normal day-to-day stimuli was observed directly each week using a scan sampling method to look for the presence or absence of a defined set of anxious behaviors (e.g., spinning, pacing, jumping, barking). In addition, each dog was subject to a formal behavioral eval-

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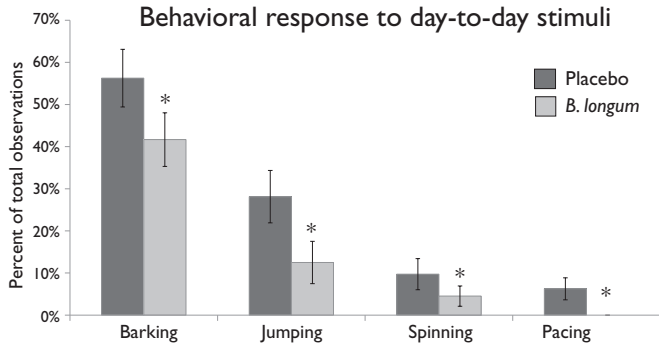
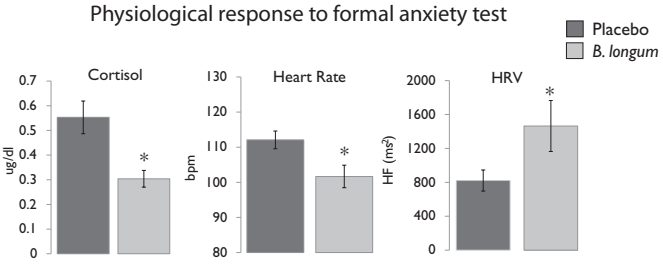
Figure 3. Behavioral change in response to day-to-day stimuli. Dogs exhibited less anxious behavior when supplemented with a proprietary strain of *Bifidobacterium longum* than when supplemented with a placebo.



* indicates a significant difference P<0.05.

Figure 4. Physiological changes in response to formal anxiety test. Dogs were less reactive (lower salivary cortisol and lower heart rate) and in a more positive affective state (higher heart rate variability (HRV)) during a formal anxiety test when supplemented with a proprietary strain of *Bifidobacterium longum* than when supplemented with a placebo.



* indicates a significant difference P<0.05.

uation to assess nonsocial, social and separation anxiety at the end of each feeding phase. Heart rate and heart rate variability were recorded through the entire formal anxiety test and salivary cortisol concentrations were assessed in response to anxiety inducing stimuli.

In response to supplementation with *B. longum*, 22/24 dogs (90%) showed an improvement in day-to-day anxious behavior including reductions in barking, jumping, spinning, and pacing as compared to when they were supplemented with a placebo (Figure 3). In addition, 20/24 dogs (83%) had reduced salivary cortisol concentrations in response to the formal anxiety test when supplemented with *B. longum* compared to when they were supplemented with the placebo. When considering cardiac activity, 18/24 dogs (75%) showed a decrease in heart rate and 20/24 dogs (83%) had an increase in heart rate variability, indicating a more positive response to the formal anxiety test when supplemented with *B. longum* compared to when they were supplemented with a placebo (Figure 4). Thus, from both a behavioral and physiological standpoint *B. longum* had an anxiolytic effect on anxious dogs.

Diet as a Tool to Help Alleviate Anxiety in Dogs

Our understanding of the impact of diet on anxious behavior in dogs is in its infancy. Although there are a handful of studies available in the literature, they are not without confounds and limitations. Two new solutions that warrant consideration are fish oil and probiotics. Using a holistic approach that incorporated both behavioral and physiological measures, Nestlé Purina has demonstrated that anxious dogs treated with either fish oil or a proprietary *B. longum* are less reactive (lower cortisol), more calm (lower HR), and potentially in a better affective state (higher HRV) when experiencing anxiety-provoking stimuli than when they were treated with a placebo. In addition, and possibly most noticeable to an owner, anxious dogs treated with either fish oil or *B. longum* exhibited less daily anxious behavior than when they were treated with a placebo. Hence, these solutions have the potential to contribute positively to a management plan for improving the well-being of anxious dogs.

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