Chapter 8

Diet matters!

You may have heard of the gut–brain axis. This phrase refers to the two-way communication between the digestive tract and the brain (gut ⇔ brain).

These two systems share information via a slew of biochemical signals, or ‘messenger molecules’, including neurotransmitters, hormones, and many different types of small molecules. Some of these molecules come directly from our food, and others from the breakdown of food by our gut microbes.

This is how we’re made; our bodies are designed to be fully integrated. We function as an integrated whole, and the entire system is affected when there is any trouble anywhere. Hence the saying, “As goes the gut, so goes the brain.”

A disordered gut, especially the resident microbes, affects mood and reactivity — “irritable bowel, irritable person” — so diet matters a great deal in highly sensitive dogs.

Perhaps I just haven’t looked hard enough, but thus far I haven’t found any studies that looked specifically at the effects of diet on reactivity in highly sensitive dogs, nor even on the effects of diet in highly sensitive people.

That baffles me, because what I eat has a huge impact on how I feel and thus on how I behave; and how I feel affects what I eat. This food ⇔ function connection has also been my experience with my own highly sensitive dog, and with a variety of animal species in my veterinary practice.

What I did find, though, is a few studies on the effects of dietary supplements on anxiety in dogs. I also found a couple of studies on high-starch diets and behavioral reactivity in horses. We’ll take a look at each of them. But first, I want to say a few words about dietary starch.

Avoid starch

Diet is so important to health and well-being that I've written an entire book about feeding dogs,[1] so I won't repeat much of it here. The overarching theme is to get as close as you can to the dog's natural (*i.e.,* ancestral) diet.

Dogs are carnivores whose nearest relatives are wolves and other wild canids (*e.g.,* dingoes), so the dog's natural diet primarily consists of animals (muscle, organs, bones, tendons, ligaments, skin, *etc.*), with lesser amounts of plants, and almost no starch.

Particularly in relation to behavioral reactivity, the single most important thing you can do is to cut way down on the amount of starchy foods or ingredients in your dog's diet.

The starchy foods or ingredients most often used in commercial dog-foods are these:

- cereal grains, or simply 'grains' — wheat, corn (maize), oat, barley, rice, sorghum, millet, rye, *etc.*
- starchy root vegetables, or 'tubers' — potato, sweet potato, yam, tapioca (from cassava or manioc), *etc.*
- legume seeds, or 'pulses' — peas, beans, lentils, lupins, *etc.*; these include soy products and chickpeas

The labels on commercial dog-foods may list these ingredients as [something] 'starch', 'flour', or 'meal', such as potato starch, pea flour, or corn meal. In relation to the dog's natural diet, they're all high-starch ingredients, even the legumes, regardless of how they've been milled or otherwise processed.

They're all potentially problematic, no matter how much spin is used when marketing the product. For example, "grain-free" foods are almost always grain-free (*i.e.,* they contain no cereal grains), but they are almost never *starch*-free. The manufacturer simply substitutes one or more of the other groups of starchy foods for the cereal grains.

Why are starchy foods used in diets for dogs? Largely because they are cheap compared with meat and other animal products, and pet-food manufacturing is a business. But there is another, even less defensible reason that I'll discuss later in relation to the protein content of the dog's diet.

I'll weave more information about starch through the next several pages as I discuss a few studies on behavioral reactivity in horses and anxiety in dogs. For now, I'll simply say this:

If in doubt, cut way down on the starchy foods or cut them out altogether for a few weeks and see what difference it makes to your dog's behavior.

You'll likely see improvements in the frequency and intensity of reactive behavior in situations you know are generally a problem.

You're also likely to see improvements in chronic health complaints such as ear infections and digestive upsets, as well as niggly little things such as a dull coat, itchy/greasy/flaky skin, anal sac problems, and the "doggy" smell.

starch and reactivity in horses

I first made the connection between starch and behavioral reactivity when I was a young vet working with racing and show horses on high-grain diets. To this day, cereal grains are widely used in equine diets, although grain-free diets based on pulses and sugarbeet pulp are now common.

To me, there was a clear connection between the amount of grain in the diet and the horse's reactivity, and it was far worse and more problematic in some horses than in others. The more grain in the diet, the more flighty, unpredictable, and difficult some horses were to handle.

In short, high-grain diets amplified underlying — which I now know to be genetically determined — behavioral sensitivity.

When the amount of grain in the diet was decreased, especially when the horse was taken off grain-based feeds altogether (*e.g.,* when 'spelling' or taking a break from training and competition), these horses would calm down and be far less reactive. Chronic health problems also improved with this single dietary change.

A couple of studies in horses have now proven this connection. Researchers at the University of Glasgow in Scotland conducted a pair of studies on mature horses[2] and young ponies[3] in which they tested behavioral responses to a strange person and to a couple of novel objects, under two different dietary conditions: a high-starch diet and a high-fiber (low-starch) diet. The novel objects, which were tested separately, were a large box covered in foil and a triangular road sign.

For reference, the horse's natural diet is high in fiber and averages less than 2% starch, seldom exceeding 4% starch.[4]

I'll focus on the pony study, as it involved 10 unhandled Welsh ponies who were about 18 months old. That's roughly equivalent in developmental stage to a dog who is 6–8 months old.

Having little experience with people, these ponies were more reactive overall than the mature and well-handled horses in the first study. The behavioral responses to diet were thus more obvious in the young ponies, not being obscured by the learned behavior and life experience of the mature horses.

Over the course of the 4-week study, all 10 ponies were fed each of the diets in what is known as a cross-over study design. This is an important point because each pony served as his or her own 'control' or baseline when comparing the effects of the two diets.

The ponies were randomly assigned to one diet for 2 weeks and then switched over to the other diet for another 2 weeks, with a 2-day (4-meal) transition period in between.

The behavioral tests were performed at the end of each diet period. That's important because it allowed the ponies' gut microbes 2 weeks to adjust to the different diet before the researchers tested the effect of diet on behavior.

The main portion of both diets was hay (< 4% starch). For the high-starch diet, a concentrate mix was also fed which contained 23% starch, such that this diet supplied 1 gram of starch *per* kilogram of body weight (1 g/kg bwt starch) *per* meal. The high-fiber diet contained half this amount of starch. We aren't told the exact amounts of hay and concentrate fed on the high-starch diet, but the total diet was probably around 7.5% starch.

That wouldn't be considered a high-grain or high-starch diet in an adult performance horse, but it was enough to increase behavioral reactivity in these young ponies.

a quick detour to talk about dogs

For comparison, the average dry dog-food contained 35–40% starch in a 2022 study that tested 10 different kibbles in the US.[5] Five foods were between 30% and 40% starch, but the rest were over 40%, and one contained a little over 46% starch.

In the same study, the 10 grain-free kibbles tested were generally lower in starch, but they still averaged around 22% starch, and ranged from 11% to 34% starch. For a carnivorous species such as the dog, all of these kibbles represent high-starch diets.

Over the millennia that dogs and humans have lived together, dogs have made some adaptations to the high-starch diets they shared with their people, particularly after certain grasses were domesticated to become cereal grains (which are grass species).

For example, compared with ancient dog breeds and with wild canids such as wolves and dingoes, modern dog breeds have more copies of the genes that code for amylase, the enzyme that begins the process of starch digestion in mammals.[6,7]

Like humans and horses, dogs produce amylase in their salivary glands and pancreas, and they have separate genes for each.

However, it is simply not true that dogs are well adapted to high-starch diets. The *activity* of salivary amylase in dogs is a tiny fraction of that in humans. This is tested in a Petri dish by measuring the zone of lysis, or enzymatic breakdown, of starch in the presence of a saliva sample.

In a study that compared salivary amylase activity in a wide variety of species, activity in domestic dogs was at least 56 times, and up to 31,800 times, lower than that in humans.[7]

Furthermore, it's not quite as simple as domestic dogs having more copies of the amylase genes than their wild counterparts. There is enormous variation in amylase gene copy numbers and expression as well as amylase activity among individuals within the same species, and even within the same breed.[6,7]

In addition, being able to digest more starch with amylase does not diminish the adverse effects of a high-starch diet. It simply shifts the harmful effects from the gut to the circulation (*e.g.*, increased incidence of obesity, type 2 diabetes, and so on).

There is always a race between the enzymatic digestion of starch by amylase and the breakdown ('fermentation') of starch by the gut bacteria and other microbes. It begins in the mouth and continues in the stomach — all before the starch makes it to the small intestine, where amylase from the pancreas goes to work on it. Any starch left over is fermented by the microbes in the colon.

As enzymatic digestion of starch is rate-limited, the more starch there is in a meal, the more the gut microbes feature in its breakdown. Microbial fermentation of starch and other rapidly fermentable carbohydrates produces organic acids, including lactic acid, and gases, including carbon dioxide (CO_2).

As a consequence:

1. The cells that line the gut and are crucial to its barrier function may be damaged.
2. The many acid-sensitive bacteria in the gut community may be inhibited or killed, with sometimes toxic results; and potentially pathogenic bacteria may overgrow.
3. Gut motility — the orderly mixing and moving of food downstream — may be disordered.

These effects are variously referred to as 'leaky gut', 'dysbiosis', and 'irritable bowel syndrome'. And given the gut–brain axis, none of it does the brain any good, as we shall see.

now back to the ponies

The main finding of the Scottish study was that when the ponies were fed the high-starch diet, they were more alert, uneasy, and reactive, and they spent less time investigating their surroundings than when they were fed the high-fiber diet.[3]

Put another way, when the ponies were fed the more species-appropriate diet (high in fiber and low in starch), they were more settled and at ease, and so they spent more time investigating their surroundings rather than being on high-alert.

Using the descriptive terms of the investigators, the ponies were perceived as being more tense, nervous, or unsure when on the high-starch diet.

Other descriptions included stressed, anxious, alert, excitable or excited, scared, worried, spooked, flighty, jumpy, on-edge, restless, shy, energetic, agitated, confused, annoyed, uptight, terrified, upset, panicked, frightened, skittish, cautious, careful, hesitant, dominant, sharp, scatty, aware, wary, afraid, and uncertain.

As for the mature, well-handled horses, their behavioral responses to the high-starch diet were similar but more muted.[2] Their heightened arousal on the high-starch diet was best seen in their heart rate responses during the behavioral tests.

The young ponies were not amenable to wearing a heart rate monitor, so we have heart rate data for just the mature horses.

Heart rate reactivity, particularly the maximum ('peak') and average heart rates, during the behavioral tests were significantly higher when the horses were fed the high-starch diet than when they were on the high-fiber, low-starch diet.

leaky gut

The pony study also examined changes in the gut bacteria between the two diets. The two measurable behaviors most strongly correlated with changes in the gut bacteria were 'pace change' (the number of times a pony changed pace among walk, trot, and canter) and time spent investigating their environment.[3]

The ponies changed pace more, indicating greater anxiety or uneasiness, and they investigated less, indicating greater wariness, when on the high-starch diet — and gut bacterial community composition was clearly associated with each of these behaviors.

When on the high-starch diet, there was a significant decrease in several groups of bacteria that break down dietary fiber, and a significant increase in lactic acid-producing bacteria, notably those in the *Streptococcus* group.[3]

So, not only would fiber digestion be inhibited, there would also be an increase in lactic acid production in the gut, which in horses is known to cause compromise of the intestinal barrier (*i.e.,* leaky gut).

The amount of starch in the high-starch diet was well below the accepted threshold for starch digestibility in horses. That term refers to the maximum amount of starch a horse can eat in a single meal without any spilling over into the large intestine. It is generally accepted as being 2 g/kg bwt starch.[8]

In these ponies, all of the starch in the high-starch diet should have been digested in the small intestine. None should have been able to pass into the large intestine, where starch is assumed to cause the most problems in horses.

> ❝ This shows that even small additions of starch to the diet, which should not pass through the small intestine undigested, can still result in an increase in *Streptococcus* bacteria. ❞ [3]

In short, and of most importance to us here, even an ostensibly "safe" level of starch for the species altered the gut microbial community in a potentially harmful way and was associated with increased behavioral arousal and reactivity.

However, the authors were at a loss to explain why the mature horses and young ponies were more aroused and reactive on the high-starch diet. They assumed that it had something to do with the high blood glucose levels that occur after a high-starch meal, and the associated insulin response.

Think of it as like the "sugar high" you get after eating a donut, followed by the inevitable "crash" as your blood glucose ("blood sugar") spectacularly rises and then precipitously falls.

That's possible, I suppose, but eating donuts does not make me anxious, uneasy, wary, and all the rest. (It makes me happy. ☺)

One possibility not even considered in these studies is a leaky gut. Disruption in the normally tight junctions between the cells of the gut lining allows molecules normally confined to the gut to enter the bloodstream. In addition, whole bacteria are able to leak out of the gut and into the bloodstream.

Here's a rather unpalatable example. An Italian study of young horses raised for meat measured the numbers of intestinal bacteria found in the lymph nodes that drain the intestine (the mesenteric lymph nodes) and in the liver.[8]

The horses on a high-grain diet (28% starch) had 2–18 times (median, 10 x) as many bacteria in their mesenteric lymph nodes, and 11–38 times (median, 25 x) as many bacteria in their liver, as the horses on a high-fiber diet (6% starch).

> ❝ The present study shows that feeding horses high amounts of cereal grains is wasteful from an economic stance and harmful from a welfare point of view. … [F]eeding horses high amounts of cereal grains can lead to a condition of increased intestinal permeability. ❞ [8]

The high-starch diet in this study supplied 4 times the threshold for starch digestion in horses, whereas the high-fiber diet remained below the threshold.

It's worth noting that this high-fiber diet was only a little lower in starch than the high-starch diet in the Scottish pony study (6% *vs* 7.5%).

Even on the high-fiber diet in the Italian study, bacteria had escaped the gut and made their way into the adjacent lymph nodes and the liver. There were 100–400 bacteria *per* gram of lymph node and 100–700 bacteria *per* gram of liver in the horses on the diet that was 6% starch.[8]

In short, and of most importance to us here, even ostensibly "safe" levels of starch for the species resulted in a leaky gut.

The presence of so many bacteria in the tissues outside the gut creates a low-grade but chronic state of systemic inflammation and oxidative stress as the immune system targets these invaders for destruction.

For example, in a study of unexplained fever in horses (termed 'fever of unknown origin'), more than 60% of cases had intestinal microbial DNA in their blood, indicating compromise of the intestinal barrier.[9]

A chronic state of systemic inflammation, or inflammatory dysregulation ('dysinflammation'), and oxidative stress makes everything in the body grumble and complain, including the brain and the rest of the nervous system.

I'll mention starch content again as I discuss the next several canine behavior studies, because in every one of these studies the dogs were fed a high-starch diet.

Nutritional supplements

Here, we do have some canine studies that are instructive, as they specifically examined anxiety and related behaviors in the dogs. I want to make two important points before we dive in, though.

First, anxiety and high sensory-processing sensitivity are not the same thing. Anxiety is common in highly sensitive dogs, but only because they are more easily overstimulated and overwhelmed than less sensitive dogs. With sensitivity-aware diet, lifestyle, and training, highly sensitive dogs are generally not anxious.

In other words, anxious dogs are not necessarily highly sensitive, and highly sensitive dogs are not necessarily anxious.

So, while the following studies may very well have included some highly sensitive dogs, we have no way of knowing how many or what proportion.

In the absence of that information, I want to focus on how the addition of antioxidant and anti-inflammatory supplements and tryptophan (an essential amino acid) affected the expression of reactivity in dogs showing signs of generalized anxiety.

Second, in each of these studies the diets were typical kibbles, all of which were high in rapidly fermentable carbohydrates.

This family of plant carbohydrates includes starch, simple sugars, oligosaccharides (*e.g.,* fructo-oligosaccharide, or FOS), and polysaccharides such as fructans, along with β-glucans, pectins, and gums. Collectively, they are also called nonfiber carbohydrates (NFC) or nitrogen-free extract (NFE).

The various plant fibers (cellulose, hemicellulose, lignin) are carbohydrates, too, but as they are indigestible to mammals and only slowly fermentable by the gut microbes, it's simplest to just call them 'fiber'.

The researchers appear to have given no thought at all to the role of starch in behavioral reactivity. In fact, they haven't even reported the starch content of the diets. I've had to estimate the nonfiber carbohydrate content from the nutritional data they do provide (protein, fat, fiber, and ash).

In each of these studies the antioxidant/anti-inflammatory blend or tryptophan was simply added to the typical highly-processed, high-carb diet, with no other substantial changes. With every one of these studies, I've wondered what might have happened if the dogs had been fed a more species-appropriate diet, which for a dog is very low in starch and most other NFCs.

There is glucose (a simple sugar) in blood; and there is glycogen (a glucose-storage molecule) in muscle, liver, and various other organs and tissues. But overall, the dog's natural diet is fairly low in carbohydrates, and it contains essentially no starch.

In my experience, switching a dog from a high-starch diet to a low- or no-starch diet, and otherwise making sure the dog is fed a species-appropriate diet of high-quality ingredients, is often enough to dramatically improve health and well-being, including a decrease or disappearance of problematic behaviors.

Also in my experience, adding antioxidant/anti-inflammatory supplements to a high-quality, species-appropriate diet doesn't usually result in the dramatic improvements we're about to see in dogs still on one of the typical high-carb diets.

In other words, the basic diet — whether good or bad; for better or for worse — has a greater influence on health and well-being than any additions we make to address particular problems.

That said, when you simply cannot make the full transition from a typical kibble diet to a completely natural diet, it's good to know that adding antioxidant/anti-inflammatory supplements may substantially reduce your dog's behavioral reactivity, at least in the short-term.

I hope, though, that you won't stop there and you'll continue to make the transition to an all-natural diet. The payoff is well worth the effort.

fish oil and polyphenols

Fish oil is a species-appropriate source of omega-3 fatty acids for dogs. The omega-3s are essential fats (*i.e.,* necessary in the diet) that have antioxidant and anti-inflammatory properties. They counterbalance the equally essential but pro-inflammatory omega-6 fatty acids.

By the way, inflammation is not inherently bad; it's a necessary part of our defense-and-repair system. It is chronic or persistent inflammation — dysregulated inflammation — that is a problem.

Polyphenols are plant molecules that are found in abundance in colorful fruits and vegetables, in fresh grass and other fresh herbage grazed or browsed by herbivores, and in colorful herbs such as turmeric and green tea. Polyphenols have potential antioxidant, anti-inflammatory, and neuroprotective effects.

Hill's study no. 1

This first study comes to us from Hill's Pet Nutrition.[10] (Yes, *that* Hill's, the makers of Science Diet.)

The study involved 40 mature Beagles belonging to the Hill's research facility in Kansas. While the dogs ranged in age from 5 to 14 years, most were over 7 years of age, and the authors referred to it as study of senior dogs.

The researchers set out to measure various molecules in the blood and feces (stool, poop) that have been linked to anxiety in other species (humans, rats, mice) and, from the little research we have, in dogs as well.

They also examined changes in the fecal bacteria associated with the key dietary intervention: fish oil and a blend of plants rich in polyphenols, added to the typical Hill's dry dog-food.

Three diets were compared:

1. Standard diet, called the 'washout' diet, as it was fed for the first month of the study and again for a month in between the two supplemented diets (called the 'washout' period in scientific parlance).

   The authors don't give us a complete ingredient list, but they do note that this diet contained 10 times more corn than the other two diets. For reference, the type of corn used in animal feeds averages around 70% starch.[4]

   In addition to corn, this diet contained chicken meal, rice, pork, corn gluten, soybean oil, carnitine, and flax seed.

   This diet appears to be the typical Hill's fare for maintenance in adult dogs. It contained only half the flax seed in the other diets and no fish oil, so its total omega-3 fatty acid content was about half that of the other diets.

2. Control diet, which is a misnomer, as it was a supplemented diet, but without the tomato pomace (see below) that was added to the 'Test' diet.

   I'm trying to keep to the language used in the study as much as possible while making this summary plain and clear.

   Again, we don't have a complete ingredient list for this food. The authors state only that this diet contained rice, pea flour, chicken, barley, oat groats (oat grain minus its fibrous coating), eggs, fish oil (0.5%), lipoic acid, and a blend of citrus pulp, carrot, and spinach designed to add polyphenols.

3. Test diet, which was the Control diet plus tomato pomace.

   Tomato pomace is the left-overs from the processing of tomatoes for human foods and condiments. It's mostly tomato skin, with some pulp and seeds. It was added to the test diet as a further source of polyphenols.

Over the 4-month study, all dogs were fed each diet for 1 month at a time, with the unsupplemented diet fed for the first month and again for the 1-month washout period in between the two supplemented diets. So, this is another cross-over study in which each dog served as his or her own ‘control’ for comparison.

We aren’t told the starch content, but all three diets contained 56% nonfiber carbohydrates, most of which will have been starch.

Not surprisingly, plasma concentrations of several molecules implicated in anxiety disorders were significantly higher when the dogs were fed the unsupplemented (standard or washout) diet. Most of them you won’t have heard of, and the biochemistry gets really complicated, so let’s not.

Their main goal appears to have been validating the addition of fish oil and their polyphenol blend as a potential means of reducing anxiety disorders in dogs, particularly senior dogs.

As I write this (early 2025), Hill’s hasn’t released an “anti-anxiety” diet for dogs; but given their broad array of problem-targeted diets, it is surely only a matter of time.

However, they do include fish oil and this polyphenol blend (with tomato pomace) in their “senior vitality” dry-food for dogs. This diet is 56% nonfiber carbohydrates, most of which will be starch.

lactic acid

One of the things that interested me most in the granular details of this study is a particular marker of starch breakdown by certain gut microbes: lactic acid, or lactate.

Lactic acid is produced by many different types of bacteria that are called ‘lactic acid-producing’ or simply ‘lactic acid bacteria’. They include, among others, *Lactobacillus, Lactococcus, Streptococcus, Pediococcus, Carnobacterium,* and *Weissella.*

The lactate concentrations in the feces and blood (specifically, the plasma) were higher after 1 month on the unsupplemented diet, which contained “> 10-fold more corn,” than after 1 month on either of the supplemented diets (Figure 1). These differences were highly significant ($P \leq 0.001$).

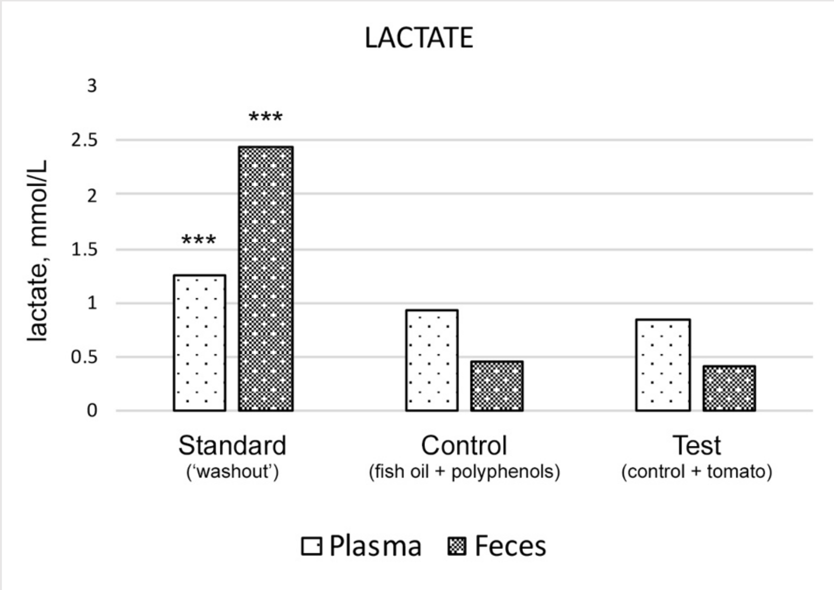

Figure 1. Lactic acid concentrations after 1 month, comparing the unsupplemented (Standard) diet and the two supplemented diets (Control and Test). *** Mean plasma and fecal lactate concentrations were significantly higher on the unsupplemented diet ($P \leq 0.001$).[10]

In mice, lactate produced by lactic acid bacteria in the gut promotes anxiety-like behaviors.[10] So, this one little data set tells us something important about high-starch diets in dogs and their effects on the gut microbes which has implications for behavioral reactivity in dogs.

serotonin

I've tried my best to keep the biochemistry to a minimum, but now I need to do a quick dive into the biochemistry of serotonin because this study is an example of "the devil in the details."

I'll keep it as clear and concise as I can, and stay focused on behavioral reactivity in dogs. Serotonin comes up again in the next couple of studies, so let's nail it down now.

This Hill's study also showed that plasma serotonin was higher after 1 month on the supplemented (Control) diet than after 1 month on the unsupplemented diet (Figure 2, next page).

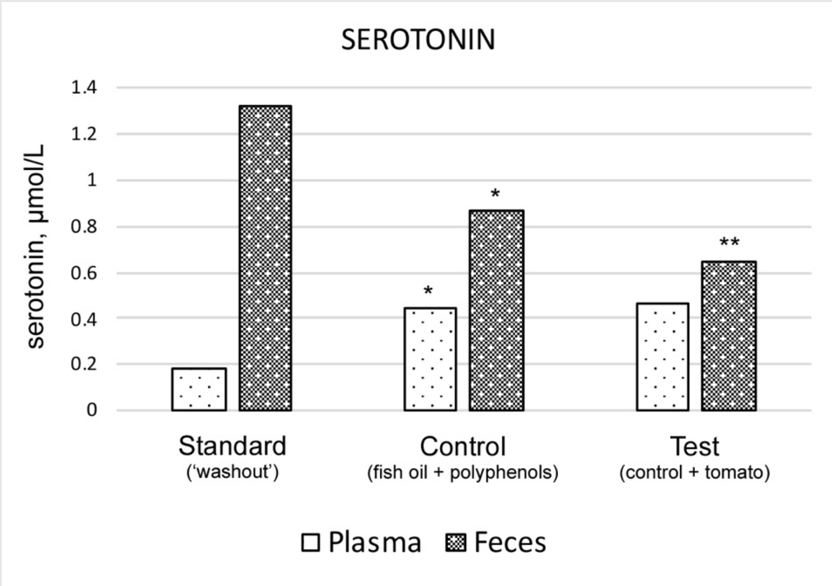

Figure 2. Serotonin concentrations after 1 month, comparing the unsupplemented (Standard) diet and the two supplemented diets (Control and Test). *, ** These means were significantly different from the unsupplemented diet (* $P < 0.05$; ** $P < 0.01$).[10]

That sounds like a good thing! Serotonin is a very important neurotransmitter that is involved in mood and emotional reactivity, so the implication is that the supplemented diet would potentially improve behavioral reactivity in dogs.

Here's a primer on serotonin as it relates to anxiety in people:[11]

> " Serotonin, often referred to as the "feel-good" chemical, is a neurotransmitter that acts as a messenger in the brain and throughout the body. It's involved in a wide range of physiological processes, from regulating sleep and appetite to influencing mood and social behavior. …
>
> Low levels of serotonin may result in:
>
> 1. Increased sensitivity to stress and anxiety-provoking stimuli
> 2. Difficulty regulating emotions and mood
> 3. Impaired ability to cope with challenging situations
> 4. Heightened fear responses and excessive worry

> [W]hile serotonin plays a significant role in anxiety, it's not the only factor. Other neurotransmitters, genetic predisposition, environmental factors, and life experiences all contribute to the development and maintenance of anxiety disorders. ❞

But there's a fatal flaw in the simplistic conclusion that the supplemented diet increased plasma serotonin, and thus may be expected to help with behavioral problems in dogs:

Serotonin does not cross the blood-brain barrier.[12]

The blood-brain barrier refers to the tight junctions between the cells that line the tiny blood vessels (capillaries) in the brain and to the sheath of cells that lie just outside these vessels.

Compared with the capillaries in other parts of the body, which are leaky by design, the blood-brain barrier is highly restrictive and highly selective in what it allows to pass from the blood into the brain. This is all to protect the critical executive functions of the brain. (It does the same for the spinal cord as well.)

Among other molecules of interest, the blood-brain barrier prevents serotonin in the bloodstream from entering the brain. Serotonin activity in the brain relies exclusively on serotonin production *within* the brain.

Serotonin is produced by several different organs and cell types. About 90% is produced in the gut, by specialized cells in the gut lining (enterochromaffin cells) and by certain gut bacteria. A measly 1–2% of the body's serotonin is produced in the brain.

But while serotonin cannot cross the blood-brain barrier, tryptophan, the amino acid that is its parent or 'precursor' molecule, can and does cross the blood-brain barrier. So does the intermediate molecule, 5-HTP (5-hydroxy-tryptophan).

Production of serotonin in any tissue, including the brain, goes like this:

tryptophan → 5-HTP → serotonin

More on tryptophan in a bit, because dogs need it in their diets. It is one of the 10 essential amino acids in dogs, meaning that they cannot make it themselves; they must get it from their food.

The serotonin that is produced in the gut is important for gut motility, secretion of digestive fluids, and absorption of nutrients. Too little or too much, and digestive disorder ensues.

Here's a personal example that runs the risk of "oversharing"...

When I eat too much of my favorite serotonin-precursor food (chocolate), I experience increased gut motility, sometimes with cramping and even diarrhea. For me, chocolate is a 'pro-kinetic' agent, increasing gut motility.

As I mentioned, serotonin is also produced by some types of gut bacteria. Collectively, our gut bacteria produce all four of the neurotransmitters that are important in mental–emotional health:[13]

- serotonin
- dopamine
- norepinephrine or noradrenaline
- GABA (gamma-amino-butyric acid)

In fact, the gut bacteria produce a vast array of molecules that have the potential to influence brain function either directly or indirectly. All of this is still being uncovered in an emerging field of research called neuro-microbiology.[13]

There's a whole lot more to be learned here, and way too many assumptions being made in the meantime.

But here's the really interesting bit: several lactic acid-producing bacteria also produce serotonin.[13]

In the Hill's study, a fascinating thing happened: serotonin was significantly higher in the bloodstream when the dogs were fed the supplemented diet, but significantly higher in the *feces* when the dogs were fed the *un*supplemented diet (see Figure 2 again).[10]

The authors didn't think that rated a mention, but to me it suggests that the unsupplemented diet, with its higher plasma and fecal *lactate* concentrations, shifted serotonin production in the gut toward the microbial population and away from the enterochromaffin cells in the gut lining.

If that's so, then it also indicates that the serotonin produced by the gut microbes was primarily eliminated in the feces. Hence the higher fecal but lower plasma serotonin concentrations when the dogs were fed the unsupplemented diet.

The biochemistry is so complicated that I've created a simple(ish) diagram on the next page so that we can refer to it as-needed. An explanation is provided on the facing page.

We aren't told the tryptophan content of the diets, but assuming that it was similar among the three diets — all made by the same pet-food company, for use in a study comparing metabolites linked to anxiety — it suggests that the pathways of tryptophan metabolism in the gut were significantly affected by diet.

This possibility has some support in the other tryptophan metabolites that were significantly different in the plasma and feces between the unsupplemented and supplemented diets. I'll spare you those details and go straight to tryptophan and its possible role in behavioral reactivity in dogs.

tryptophan and behavior

You may be going cross-eyed by now, but please bear with me, because things are about to get ... maddening — but in a good way, because we're about to examine the conventional rationale for feeding dogs a high-carb, low-protein diet.

As I mentioned earlier, tryptophan is one of the essential amino acids in dogs. Dogs must consume tryptophan in their diets, as they cannot make it themselves. Recommended daily intakes of tryptophan for maintenance in adult dogs are shown in Table 1 on page XX.

It's worth noting that the dog's natural diet, which primarily consists of animal products, is rich in tryptophan.

For example, the recommended daily intake of tryptophan for a 10-kg or 22-lb adult dog is 260 mg.[14] The dog would get all that from just over 2 ounces of cooked boneless, skinless chicken breast, or from 1 raw chicken thigh or drumstick.[15]

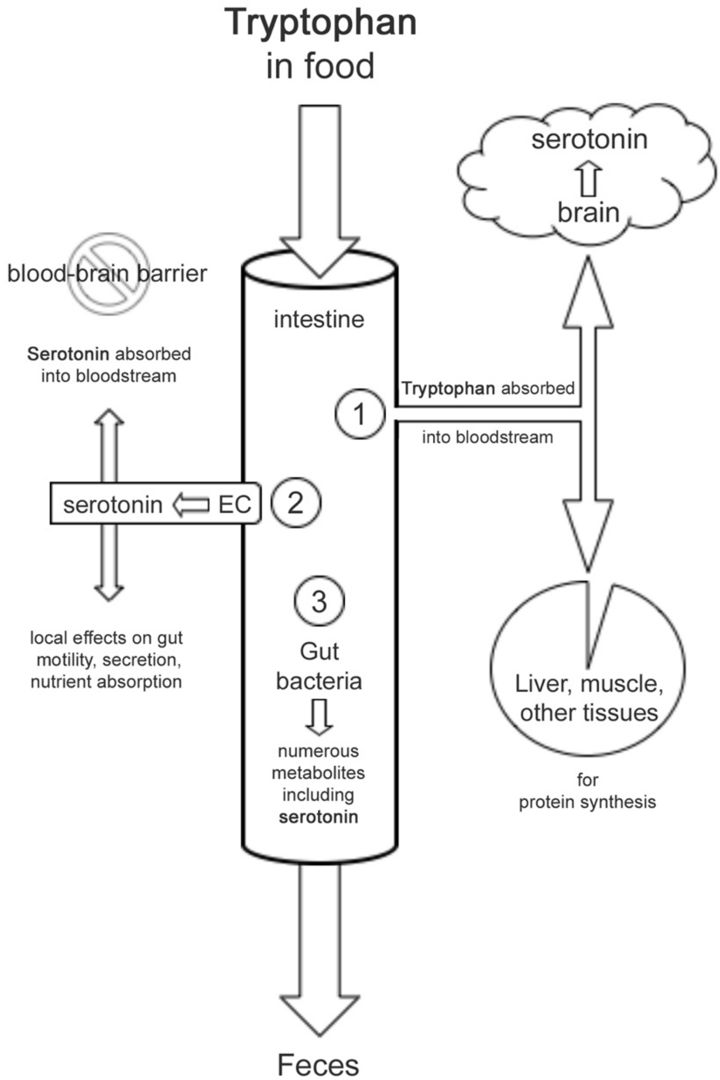

Figure 3. Three main pathways of dietary tryptophan metabolism in the gut after a meal. Tryptophan is one of the essential amino acids in dogs. Among its other uses in the body, it is required for serotonin production in the brain. The numbers and abbreviations are explained on the facing page.

Figure 3 illustrates the three main pathways of tryptophan metabolism after a meal.

1. Tryptophan is absorbed from the small intestine directly into the bloodstream, as with all amino acids.[16,17]

   Tryptophan is able to cross the blood-brain barrier, where it is used to produce serotonin in the brain.

   In all other tissues, but especially in the liver and muscles, tryptophan is used by the cells for protein synthesis.

   Insulin facilitates the absorption of amino acids by the liver, muscles, and other tissues, so indirectly it may decrease tryptophan availability to the brain.

   Cortisol does the opposite, indirectly increasing tryptophan availability to the brain.

2. Tryptophan is absorbed by the enterochromaffin (EC) cells in the intestinal lining. There, it is converted first to 5-hydroxy-tryptophan (5-HTP) and then to serotonin.[16,17]

   Most of that serotonin is used locally to regulate gut motility, secretion of digestive juices, and nutrient absorption.

   Some of it is absorbed into the bloodstream, where it can easily be measured.

   Importantly, serotonin in the bloodstream does not cross the blood-brain barrier, although 5-HTP can.

3. Tryptophan is metabolized by the gut bacteria into various bioactive molecules, including serotonin. The gut bacteria also produce dopamine, norepinephrine (noradrenaline), GABA (gamma-amino-butyric acid), and other molecules that can affect brain function directly or indirectly.[13,17]

   While these molecules undoubtedly have local effects in the gut, at least some, and perhaps even most, are eliminated in the feces, rather than being absorbed into the bloodstream.

Table 1. National Research Council (NRC) recommended daily intake of tryptophan for maintenance in healthy adult dogs.[14]

Body weight[a]		Tryptophan[b]
kilograms	pounds	
10 kg	22 lb	260 mg
20 kg	44 lb	437 mg
30 kg	66 lb	592 mg
40 kg	88 lb	735 mg
50 kg	110 lb	869 mg
60 kg	132 lb	996 mg
70 kg	154 lb	1,118 mg

[a] This table uses the dog's metabolic weight, so the relationship is more complex than *x* milligrams (mg) of tryptophan *per* kilogram (kg) of body weight. The formula is 46.2 mg tryptophan *per* ($kg^{0.75}$).

[b] Total daily intake of tryptophan from all dietary sources, including supplements.

Proctor & Gamble | Mars Pet Care (makers of Pedigree, Royal Canin, Iams, Orijen, Eukanuba, *etc.*) claim that these figures underestimate the needs of some dogs. They advise adding purified tryptophan during manufacture, as it has higher digestibility than natural protein sources. However, they were testing a junk-food diet that was 54% corn starch, 15% chicken fat, and only 7% chicken meal.[18]

Now let's talk about tryptophan and aggression in dogs.

Understandably, one of the behavioral problems in dogs that have received the most research attention is aggression, particularly aggression toward people.

While aggression is not a characteristic of highly sensitive dogs — I repeat, highly sensitive dogs are not, by nature, aggressive — this research may still be useful to us in understanding how diet may affect behavioral reactivity in highly sensitive dogs.

For example, a study at Cornell University in upstate New York found that the concentrations of a serotonin metabolite and a dopamine metabolite in the cerebrospinal fluid were 1.5 and 1.7 times lower in dominance-aggressive dogs than in dogs with no history of aggression.[19]

The cerebrospinal fluid, or CSF, bathes the brain and spinal cord, so it is useful for measuring the products of brain metabolism separate from what may be going on in the bloodstream. That makes this study uniquely useful to us, as almost all other canine studies measured the various neurotransmitters and hormones in the bloodstream.

The concentrations of these two products of serotonin and dopamine metabolism were lowest in the subgroup of aggressive dogs who had a history of biting without warning, which the authors noted is suggestive of decreased impulse control.

Incidentally, the researchers also measured a norepinephrine or noradrenaline metabolite in the CSF. There were no significant differences between aggressive dogs and dogs with no history of aggression.

The implications of this study are that aggression and poor impulse control in dogs are associated with disorders of serotonin and dopamine production or turnover in the brain, but not of norepinephrine production/turnover. Unfortunately, there was no mention of the dogs' diets.

Similarly, a Turkish study found that the average serotonin concentration in the bloodstream (specifically, in the serum) was almost 3 times lower in aggressive dogs than in dogs with no history of aggression.[20]

I haven't found anything that clearly defines the relationship between blood and brain serotonin concentrations. Perhaps there is no clear relationship because it depends so much on the diet and everything else that's going on in that body at that time.

In other species, tryptophan transport across the blood-brain barrier and its conversion to serotonin in the brain are affected by age, breed, gender, social status, activity, and level of arousal or sympathetic nervous system activation.[16,21] In other words, brain serotonin appears to have a production- or metabolism-on-demand element to it.

Still, this Turkish study does show that there is a relationship between serum serotonin concentration and this particular reactive behavior in dogs. But again, no mention of the dogs' diets. I'm sensing a pattern here ...

I then examined a review paper on the impact of nutrition on canine behavior.[21] "At last we're getting somewhere!" I thought. Someone has been taking a comprehensive look at diet in relation to behavior in dogs. But I was to be disappointed.

When I traced the data of interest back to its source — a seminal study that looked at tryptophan and behavioral disorders in dogs — I found it rife with junk foods and manipulated statistics.

In other words, it was junk science, funded by a company that makes junk dog-food.

Let's take a good look at this study, because it appears to be committing, or at least repeating, the original sin regarding the concept that high-protein diets contribute to aggression in dogs. It also provides commercial pet-food makers with their (dubious) justification for feeding high-carb, low-protein diets to dogs.

Hill's study no. 2

This study was conducted at Tufts University in Massachusetts, at the vet school's behavior clinic.[22] No funding or conflict-of-interest statements were provided, but one of the authors worked for Hill's Pet Nutrition, which supplied the four test diets.

In all, 33 dogs completed the study, 11 dogs each in these three main groups: dominance aggression, territorial aggression, and hyperactivity.

While feeding the assigned diet, the owner was instructed to give their dog a daily score, rated from 0 (none) to 10 (extreme), for each of the following five behaviors:

- dominance aggression, particularly toward family members
- territorial aggression toward strangers
- fearfulness toward any strange person, situation, or experience

- hyperactivity
- excitability

Oh, and fortunately for us, the researchers also measured plasma tryptophan and serotonin concentrations at the end of each week the dog was on the assigned diet.

Four diets were tested, all dogs eating each diet over the course of the study for 1 week at a time, with a transition period of at least 3 days between each diet. This was another cross-over study, with each dog serving as his or her own ‘control’.

The four diets, with their relevant nutrient profiles, are shown in Table 2 on the next page.

All four diets were at least 50% nonfiber carbohydrates, most of which will have been starch. Essentially, the researchers just switched around the first few ingredients, tinkered with the fat and fiber content, and added tryptophan to two of the diets.

Trouble is, it made no difference. There were no significant changes in any of the five behaviors, in any of the three groups of dogs, on any of the four test diets. None.

So, the researchers went back to the drawing board and changed their analytical plans. Instead of focusing on the effects of diet on the problematic behavior within each group, such as a change in dominance-aggression in the dominance-aggressive dogs or a change in hyperactivity in the hyperactive dogs, they panned out and examined all 33 dogs as a single group.

For example, they now considered the scores for dominance-aggression in the hyperactive dogs, and scores for hyperactivity in the dogs with dominance-aggression.

This going back and re-analyzing your data, hoping to get results you like better, is known as *post hoc* (after-the-fact) analysis, data mining, data dredging, data contortion, data torture, and *P* hacking. And it’s a big, bright, flapping red flag.

The second statistical sin they committed was failing to adjust their threshold for significance to account for the multiple comparisons they subsequently ran.

Table 2. Nutrient profiles of the four diets (low- or high-protein, with or without added tryptophan) fed in the second Hill's study.[22]

	LP	LP + T	HP	HP + T
Protein	19%	19%	31%	31.5%
Fat	17%	17.5%	12%	11%
Fiber	8%	7%	1.5%	1.5%
Carbs	51%	51.5%	50%	50.5%
Added T	0	1.45 g/kg food	0	1.45 g/kg food
Total T	0.18%	0.3%	0.24%	0.37%
Daily T intake[a]	305 mg	499 mg	425 mg	668 mg
% of RDI[b]	117%	192%	164%	257%

LP: low-protein diet. The first three ingredients were corn, poultry meal, and corn starch. [mg, milligrams; g, grams; kg, kilograms]

LP+T: low-protein diet + tryptophan (T).

HP: high-protein diet. The first three ingredients were poultry meal, corn starch, and corn.

HP+T: high-protein diet + tryptophan.

[a] Daily tryptophan intake, calculated from the reported feeding rate.

[b] Amount of tryptophan consumed as a percentage of the recommended daily intake (RDI) for maintenance in adult dogs (shown in Table 1).

Either of these *faux pas* increases the likelihood of false-positive results when significance is kept at a threshold of $P < 0.05$. (See page XX for an explanation of this number.)

I don't know how many comparisons they ended up running, but they report on at least 30, so the threshold should have been set at $P < 0.0017$, and probably even lower.

When you keep the threshold at $P < 0.05$ while running 30-plus comparisons, the likelihood of getting at least one false-positive result increases from less than 5% to almost 80% — and there's no way of knowing which of your results is unreliable.

Trouble is, their *P* hacking made very little difference. All they found, using this corrected threshold for statistical significance, was that addition of tryptophan to the high-protein diet dropped the average dominance-aggression score by 0.8 point on an 11-point scale (0–10, including 0) compared with the high-protein diet without added tryptophan.

The average dominance-aggression score in all 33 dogs on the high-protein diet was 1.8 out of a maximum of 10 points. It dropped to 1.0 when tryptophan was added. How spectacularly underwhelming.

In comparison, all 11 dogs in the dominance-aggression group had scores of > 2 at the start. The scores for the specific behavior groups are not reported, so we have no idea what the average score was for these dogs, nor how high their scores went.

Dogs in this group reacted aggressively toward family members in at least 5 of 30 dominance-aggression-promoting situations and they showed some aggressive behavior at least 5 days/week. Again, diet made no difference to aggression scores in these dogs.

Of particular relevance to highly sensitive dogs, there was no effect of dietary protein or tryptophan content on fearfulness, hyperactivity, or excitability. None. Not even after the statistical shenanigans.

The researchers also reported on the ratio of tryptophan to a group of much more plentiful amino acids collectively called the large, neutral amino acids (LNAAs). They include tyrosine, phenylalanine, leucine, isoleucine, and valine.[21]

I'm not going to spend much time on this detail, except to note that the Tryp:LNAA ratio of the four diets, which ranged from 1:14 to 1:25, had no effect on behavior scores.[22] None.

Experimentally, the LNAAs compete with tryptophan to cross the blood-brain barrier. In theory, the higher the LNAA content in relation to tryptophan, the lower the tryptophan content in the brain.[21] If that's the case in dogs as well, then a ratio greater than 1:25 is needed for an observable behavioral effect, based on this study.

Before we leave this study to its dusty pedestal, there were no significant effects of diet on plasma tryptophan or serotonin concentrations in any of the behavior groups, not even at the threshold of $P < 0.05$. None.

That may explain why there were no significant effects of diet on behavior without some, er … "creative accounting."

Another possible explanation is that even their low-protein diet, without any added tryptophan, exceeded the recommended daily intake of tryptophan by almost 20%. As these dogs were fed four different junk-food diets, this study suggests that it is quite difficult to create a tryptophan-deficient diet for dogs.

Comparing apples to apples, the high-protein diet in this study contained only 17% more protein but 22% less tryptophan than a popular commercial raw dog-food in the US whose first three ingredients are chicken (including ground bone), chicken liver, and chicken heart.[23]

My point is that a commercial diet which hews closely to the dog's natural diet more-than meets a dog's daily requirements for both protein and tryptophan. In fact, that raw diet exceeds by wide margins the recommended daily intake for protein and tryptophan in dogs, without having to add tryptophan. And it is low in nonfiber carbohydrates (10%).[23]

Take another look at Figure 3 on page XX. The fact that neither of the two high-carb, tryptophan-supplemented diets increased the plasma tryptophan or serotonin concentrations in this second Hill's study suggests that the added tryptophan was being used by the gut microbes, and not by the dog.

polyphenols

As for the polyphenol portion of the supplemented diets in the first Hill's study,[10] not all polyphenols cross the blood-brain barrier. For example, the polyphenols in green tea and turmeric do not, whereas resveratrol and quercetin do.[24]

In animal studies (rats, mice, and the occasional gerbil), various polyphenols help protect or restore the integrity of the blood-brain barrier and improve brain blood flow in experimental models of stroke, Parkinson's disease, Alzheimer's disease, and age-related memory loss.[24]

So, while not all polyphenols cross the blood-brain barrier, they may not need to in order to exert their beneficial effects on the brain and its functions.

The indirect effects of polyphenols on the brain are the same as those in other tissues: improved vascular (blood vessel) integrity and blood flow, enhanced antioxidant and anti-inflammatory environment, and a more beneficial profile of gut bacteria.[24]

Given the presumptively high-starch diets in the first Hill's study, and the higher fecal and plasma lactate concentrations when the dogs were fed the unsupplemented diet, it's not a stretch to conclude that these high-starch diets may have set the stage for greater behavioral reactivity, as was seen in the pony study.[3]

But how much of an effect can we expect from the fish oil and polyphenol components of the supplemented diets when the same ol' high-starch diet is fed?

Perhaps quite a bit ...

nutraceuticals

'Nutraceutical' is a mash-up of 'nutrient' and 'pharmaceutical'. It is a marketing term for a food or dietary supplement that is purported to have drug-like effects. In the US, nutraceuticals straddle a few different regulatory categories, depending on their ingredients: drug, dietary supplement, food ingredient, or food.

The next two studies of a nutraceutical-supplemented diet were both conducted by researchers at the University of Sassari in Italy. They are companion studies, so I'll discuss them together.

The test diet in both is a commercial product that is sold in the US under the brand name Forza10, whose parent company in Italy is Sanypet. The company co-sponsored or supplied the supplemented diet in both studies, and company employees are listed as co-authors on both papers.

neurotransmitters and oxidative stress

The first study involved 69 dogs of various breeds, all with behavioral disorders related to anxiety and chronic stress.[25] The average age was around 3 years, and most dogs were between 18 months and 5 years of age.

The dogs were randomly assigned to receive either the control diet or the nutraceutical-supplemented diet for 45 days. This was not a cross-over study; 34 dogs were fed the control diet and the other 35 dogs were fed the nutraceutical diet.

The control diet is not specified; it is simply described as a commercial kibble ("industrially produced"). Given its nutrient analysis and its nonfiber carbohydrate content of around 55%, it sounds like the typical grain-based kibble, similar to the standard diet in the first Hill's study, but with fish oil.

Both the control and nutraceutical diets had an omega-6 to omega-3 ratio of 0.78 to 1; or, if you prefer, 1 to 1.28, meaning that there were more omega-3 than omega-6 fatty acids in each diet.

The nutraceutical diet also is not detailed. The authors simply note these additions: pomegranate, valerian, rosemary, linden flower, hawthorn, green tea extract, and L-tryptophan (or simply tryptophan).

I looked at the US Forza10 website to see what's in their Behavior diet, which appears to be the nutraceutical diet tested in these studies. It is marketed as being "designed for dogs with behavior issues" and as helping to "stop anxiety, fear, constant barking, separation anxiety, obsessive paw licking, and inappropriate urination or defecation." [26]

The ingredients listed for this commercial product are ground rice, hydrolyzed fish protein, fish oil, anchovy meal, brewer's yeast, dried beet pulp, minerals [listed], vegetable oil, hydrolyzed potato protein, dried yeast, fructo-oligosaccharides (FOS), vitamins [listed], choline, linden flowers, DL-methionine, pomegranate, L-tryptophan, tea extract, valerian root, yucca schidigera extract, and rosemary extract.

So, the most abundant ingredient is rice! We're off to a good start. (Now, where's that 'sarcasm' emoji …)

Their main goal was to measure the concentrations of several neurotransmitters and hormones associated with behavioral disorders, and also some markers of oxidative stress, in the bloodstream before and after the dogs had been on their assigned diet for 6 weeks.

The control diet resulted in no significant changes in any of the measured substances, whereas the dogs on the nutraceutical diet showed these significant changes (averages for the group):

- serotonin, 3-fold increase
- dopamine, 2-fold increase
- β-endorphin, 4.5-fold increase
- noradrenaline (norepinephrine), 47% decrease
- cortisol, 44% decrease
- derivatives of reactive oxygen metabolites (dROMs), 23% decrease

(In fairness, these researchers did account for the multiple comparisons they ran in determining an appropriate level of statistical significance.)

As I noted in the previous section, serotonin does not cross the blood-brain barrier, so the higher concentration of serum serotonin in the dogs on the nutraceutical diet would have done them no good in terms of brain serotonin production.

It is possible that an increase in brain serotonin would have accompanied the increase in serum serotonin, as both are dependent on the same factor: dietary tryptophan content. However, we have no way of confirming that in this study, as serum tryptophan was not measured.

But in the second Hill's study, adding tryptophan (1.45 grams *per* kilogram of food) to either the low-protein or the high-protein diet made no difference to the plasma tryptophan concentration, nor to the plasma serotonin concentration.[22]

In the Italian study, there was very little additional tryptophan in the nutraceutical blend: 329 milligrams *per* kilogram of food, which on its own would supply only 23% of the recommended daily intake of tryptophan.

We know nothing about the total tryptophan content of either diet. All we're told is the amount in the nutraceutical blend. But as I noted earlier, it appears to be quite difficult to create a tryptophan-deficient diet for dogs, so both the control and nutraceutical diets probably contained adequate tryptophan.

What these results suggest is that the control diet favored tryptophan metabolism *by the gut microbes,* in the process diverting dietary tryptophan away from conversion to serotonin in the gut lining, and thus serotonin entry into the bloodstream.

As you'll recall, that appears to have been what happened in the first Hill's study (Figures 1 and 2) when the dogs were fed the unsupplemented diet.[10]

As we saw in the second Hill's study, adding tryptophan to a high-carb diet had no significant effect on behavioral problems. And as the two equine studies showed, simply increasing the starch content of the diet increases behavioral reactivity.

As for dopamine, β-endorphin, and noradrenaline, none of these neurotransmitters or hormones cross the blood-brain barrier either,[27] so we must look elsewhere for an explanation as to how this nutraceutical diet might modify behavioral reactivity in dogs.

The more likely explanation is the significant decrease in serum cortisol and the particular marker of oxidative stress (dROMs). Serum cortisol does feed-back to the brain as part of the well-orchestrated hypothalamic–pituitary–adrenal gland axis, which is upregulated as part of the body's stress response.

It is unclear which components of the nutraceutical blend are responsible for those reductions. Suffice it to say that lowering serum cortisol and oxidative stress are both potentially useful in highly sensitive dogs.

However, there are plenty of better ways to achieve these goals than with a high-carb diet that has some nutraceuticals thrown in to counter the stress-inducing effects of the high-carb diet! Feeding a species-appropriate, minimally processed diet of high-quality ingredients is the better way to go.

behavior

The companion study involved 24 dogs of various breeds, all with generalized anxiety.[28] Again, the average age was 3 years, and most dogs were between 18 months and 5 years of age.

The dogs were randomly assigned to receive either the control or the nutraceutical-supplemented diet for 10 days. As before, this was not a cross-over study; 12 dogs were fed the control diet and the other 12 dogs were fed the nutraceutical diet.

The dogs also received unspecified desensitization (counter-conditioning) and behavior modification therapy.

Once again, neither diet is detailed, but both appear to be similar to, if not the same as, those fed in the first study.

At the start and at the end of the 10-day diet trial, a certified veterinary behaviorist scored the behavioral and clinical conditions of each dog.

The behaviors evaluated included marking, anxiety, shyness (termed ‘diffidence’ in this study), irregular biorhythm (*e.g.,* sleep disturbance, variable heart rate), reactivity, restlessness (termed ‘activation’), irritability, alertness, vigilance (termed ‘constant environmental exploration’), and attention seeking.

The physical abnormalities included dandruff, itchiness, redness, seborrhea (greasy coat), dull coat, vomiting, diarrhea, flatulence, lachrymation (excessive tear production), and full anal sacs.

Behavioral and physical items were each scored as follows: 1, absent; 2, moderate; and 3, severe.

Throughout the 10-day trial, the dogs were kept in individual outdoor pens of 20 m^2 (215 ft^2). As you might expect, many were very anxious or restless on day 1. (A supplementary video accompanying this paper is illuminating.)

There were no significant behavioral changes in the dogs on the control diet, whereas the dogs on the nutraceutical diet showed a significant decrease in activity, measured by a movement sensor worn on the collar, and a significant increase in rest time that averaged an additional 2 hours/day.

In the nutraceutical group, all of the evaluated behaviors except marking significantly decreased, by between 0.7 and 1.3 point on the 3-point scale.

The most significant decreases were in the scores for anxiety, irregular biorhythm, reactivity, irritability, and vigilance. The only behavior that was not significantly affected was marking.

In addition, time spent barking significantly decreased, from an average of 3 minutes/day to 1 minute, 15 seconds/day. That's a 58% decrease in time spent barking, although 3 minutes/day is not exactly representative of problematic barking in dogs.

There were no significant physical changes in the dogs on the control diet, whereas all of the evaluated physical conditions significantly improved, by between 0.5 and 1.3 point, in the dogs on the nutraceutical diet.

The most significant improvements were in the scores for dandruff, vomiting, diarrhea, and full anal sacs.

What might these studies have uncovered if the dogs had been fed a low-starch and otherwise species-appropriate diet instead of, or in addition to, the nutraceutical blend?

At least we know that this particular blend of nutraceuticals may decrease reactivity in dogs with generalized anxiety who are fed a typical high-carb kibble.

omega-3 fatty acids and behavioral reactivity

Here is one last look at diet and behavioral reactivity in horses. A Canadian study examined the effects of two different plant sources of omega-3 fatty acids on behavioral reactivity in horses: camelina (a seed oil) and marine algae.[29] Horses being terrestrial herbivores, camelina oil is the more species-appropriate source of omega-3 fatty acids for them.

This study involved 30 young horses, all between 7 months and 6 years of age. The average age was just under 2 years, but 63% were ≤ 1 year of age. They were a good cross-section of healthy, young sport horses in North America, in terms of breed, diet, housing, and training status. Almost all diets contained at least some cereal grains.

The horses were assigned to receive (a) camelina oil, (b) a mix of camelina (75%) and marine algae (25%) oil, or (c) no oils, added to their usual diet for 6 weeks. No other changes were made to their diets or routines.

The camelina oil contained 1.6 times as much omega-3 as omega-6 fatty acids, while the combo oil, with its marine algae component, contained 2.4 times as much omega-3 as -6.

The assigned oil was fed at a rate of 0.37 g/kg bwt/day, which works out to an average of 150 ml (5.3 oz) of oil *per* day. For a 50-lb dog, that would be just under 2 teaspoons/day.

The supplemented diets contained an estimated 1.85% omega-3 rich oil, plus whatever was in the rest of the horse' diets: various processed feeds, most of which contained flaxseed meal or oil; and spring pastures, which are rich in omega-3 fatty acids[31] and polyphenols.[32] Those in the first Hill's study contained 0.5% fish oil. The oils in this study also supplied higher omega-3 to -6 ratios than the diets in the nutraceutical studies I just discussed.

Before and at the end of the 6-week trial period, the horses underwent a novel-object test, in which either an inflatable dolphin or a pup tent was placed in the test area with the horse. Behavioral reactions, heart rate (HR), and heart rate variability (HRV) were assessed before, during, and shortly after each test.

Even after 6 weeks of supplementation, there were no significant differences in behavioral reactivity, HR, or HRV among the three treatment groups. Addition of neither oil made any difference.

Plasma fatty acids were also measured before and at the end of the 6-week diet trial. There was a significant increase in plasma omega-3 fatty acids in the horses on the combo oil (mean, 5.2% of total lipids) compared with the other two groups (mean, 3.2–3.6% of total lipids; $P < 0.01$), which is a 44–62% increase.

The main omega-3 in camelina oil is alpha-linolenic acid (ALA), but it is not efficiently converted to the more bioactive omega-3s, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in horses.[30] These latter two omega-3s are predominant in fish oil and in marine algae (which is where the fish get theirs). In fact, plasma DHA was significantly higher in the horses on the combo oil (mean, 1.47% total lipids; $P < 0.01$) than in those on the camelina oil (0.28%) or on no oil (0.23%).

Given that the anti-inflammatory properties of EPA and DHA are substantially higher than of ALA,[30] supplementation with pure marine algae oil might have been the more useful strategy.

Another possibility we must consider is that omega-3 fatty acid supplementation, on its own, has little or no effect on arousal and behavioral reactivity, no matter the source or the amount.

Organic where possible

This last dietary strategy is also important, although admittedly a challenge for most of us at least some of the time, or for at least some foods.

There are several reasons to make the effort, all centered on avoiding chemical residues that are common in conventionally raised animals and plants. They include herbicides, pesticides, vaccines, hormones, antibiotics, heavy metals, and 'forever chemicals' such as PFAS (per- and poly-fluoro-alkyl substances).

A study by the same Italian researchers who conducted the nutraceutical studies makes a good case that covers antibiotic residues and nanoparticle contaminants — something I hadn't even considered an issue.

The study involved 8 dogs, all on typical kibble diets, and all with signs of food intolerance, including chronic ear infections, diarrhea, dermatitis, and generalized anxiety.[29]

Within 2 weeks of switching the dogs to an organic, chicken-based diet, there were significant improvements in all signs of food intolerance, including anxiety. Diarrhea improved in just

48 hours, whereas the other signs took longer to resolve, ranging from 2 to 15 days.

Blood samples taken before and after the 2-week diet trial found oxytetracycline, a commonly used antibiotic in conventional food animal production, in the dogs' blood.

However, after 2 weeks on the organic diet, the average serum oxytetracycline concentration had decreased by 90%. The remainder, still measurable in the dogs' blood on day 15 of the organic diet, was thought to result from the slow release of oxytetracycline that was bound in the dogs' bones, as this antibiotic is wont to do.

Microscopic and chemical analysis of the original kibble diets found oxytetracycline and two types of nanoparticle aggregates: calcium and phosphorus (probably from ground bone), and calcium, aluminium, and silicon (probably from the mechanical processes used to make the kibble).

The authors discussed the potential for these nanoparticles to compromise the intestinal barrier (*i.e.,* cause leaky gut) and trigger food intolerances and even inflammatory bowel disease.

✻✻✻

Simple summary

I could go on (and on and on …). But let's wrap up this chapter with a simple, practical summary.

While it may be difficult at first, base your dog's diet on a wide variety of meats and veggies (avoiding the starchy ones). Include lots of organ meats (heart, liver, kidney, tripe, gizzard, *etc.*).

These days, there are some very good fresh-frozen raw dog-foods available in pet stores, feed or produce stores, and directly from the manufacturer, so they're a good place to start.

If you're inclined to be making your dog's food yourself, then please consider getting a copy of *Feeding Miss Lilly,* the book I wrote on how I fed my own dog.[1]

In the short-term, avoid any foods you know from experience cause flare-ups of digestive, skin, anal sac, or ear problems in your dog. Whether or not it's obvious, those physical issues are usually accompanied by an increase in behavioral problems, including greater reactivity.

In time, you may be able to feed these items occasionally without any problems, but until your dog is on an even keel, it's generally best to avoid them.

Also avoid highly processed foods, which include kibble and canned foods, other than as the occasional naughty treat or for emergencies. For example, keep a can of dog food in the cupboard for whenever you run out of good food.

Foods containing synthetic colouring agents, flavor enhancers with no nutritional value, and the gel-like thickener carrageenan are also best avoided, as they too can cause gut inflammation and irritability.

Lastly, give the gut microbes several days to adapt by making big dietary changes *gradually*. Respect the gut microbes or pay the price (which ain't pretty!).

References

1. King C. *Feeding Miss Lilly: on feeding dogs a great, nature-inspired diet,* revised edition. Anima Books, Emerald, VIC; 2022.
2. Bulmer L, McBride S, Williams K, *et al.* The effects of a high-starch or high-fibre diet on equine reactivity and handling behaviour. *Applied Animal Behaviour Science,* 2015; 165: 95–102.
3. Bulmer LS, Murray J, Burns NM, *et al.* High-starch diets alter equine faecal microbiota and increase behavioural reactivity. *Scientific Reports,* 2019; 9: 18621.
4. Common feed profiles. Equi-Analytical Laboratory Services, Ithaca, NY. Database on the internet, available at equi-analytical.com/common-feed-profiles/.
5. Perry EB, Valach AA, Fenton JM, *et al.* An assessment of starch content and gelatinization in traditional and non-traditional dog food formulations. *Animals,* 2022; 12(23): 3357.
6. Arendt M, Cairns KM, Ballard JWO, *et al.* Diet adaptation in dog reflects spread of prehistoric agriculture. *Heredity,* 2016; 117: 301–306.
7. Pajic P, Pavlidis P, Dean K, *et al.* Independent amylase gene copy number bursts correlate with dietary preferences in mammals. *eLife,* 2019, 8: e44628.
8. Raspa F, Dinardo FR, Vervuert I, *et al.* A fibre- vs. cereal grain-based diet: which is better for horse welfare? Effects on intestinal permeability, muscle characteristics and oxidative status in horses reared for meat production. *Animal Physiology and Animal Nutrition,* 2022; 106(2): 313–326.
9. Sun Y, Yu YT, Castillo XO, *et al.* Investigation of the blood microbiome in horses with fever of unknown origin. *Veterinary Medicine and Science,* 2025; 11: e70272.

10. Ephraim E, Brockman JA, Jewell DE. A diet supplemented with polyphenols, prebiotics and omega-3 fatty acids modulates the intestinal microbiota and improves the profile of metabolites linked with anxiety in dogs. *Biology,* 2022; 11: 976.

11. Neurolaunch editorial team. The complex relationship between serotonin and anxiety: understanding the connection. [Article on the internet] Available at neurolaunch.com/serotonin-and-anxiety/. Published 29 July, 2024.

12. Young SN. How to increase serotonin in the human brain without drugs. *Journal of Psychiatry and Neuroscience,* 2007; 32(6): 394–399.

13. Miri S, Yeo J, Abubaker S, *et al.* Neuromicrobiology, an emerging neurometabolic facet of the gut microbiome? *Frontiers in Microbiology,* 2023; 14: 1098412.

14. *Ad Hoc* Committee on Dog and Cat Nutrition. Protein and amino acids. In: *Nutrient Requirements of Dogs and Cats.* National Research Council (NRC) of the National Academies, National Academies Press, Washington DC, 2006; pages 111–137.

15. Nutrient Ranking Tool; nutrient to rank: tryptophan, all food groups, household serving size. Web database at My Food Data. Available at tools.myfooddata.com/nutrient-ranking-tool/tryptophan/. Accessed 15 January 2025.

16. Palego L, Betti L, Rossi A, *et al.* Tryptophan biochemistry: structural, nutritional, metabolic, and medical aspects in humans. *Journal of Amino Acids,* 2016; 2016: 8952520.

17. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host & Microbe,* 2018; 23(6): 716–724.

18. Templeman JR, Mansilla WD, Fortener L, *et al.* Tryptophan requirements in small, medium, and large breed adult dogs using the indicator amino acid oxidation technique. *Journal of Animal Science,* 2019; 97: 3274–3285.

19. Reisner IR, Mann JJ, Stanley M, *et al.* Comparison of cerebrospinal fluid monoamine metabolite levels in dominant-aggressive and non-aggressive dogs. *Brain Research,* 1996; 714: 57–64.

20. Çakiroğlu F, Meral Y, Sancak AA, *et al.* Relationship between the serum concentrations of serotonin and lipids and aggression in dogs. *Veterinary Record,* 2007; 161: 59–61.

21. Bosch G, Beerda B, Hendriks WH, *et al.* Impact of nutrition on canine behaviour: current status and possible mechanisms. *Nutrition Research Reviews,* 2007; 20: 180–194.

22. DeNapoli JS, Dodman NH, Shuster L, *et al.* Effect of dietary protein content and tryptophan supplementation on dominance aggression, territorial aggression, and hyperactivity in dogs. *Journal of the American Veterinary Medical Association,* 2000; 217: 504–508.

23. Instinct Raw Longevity Cage-Free Chicken Recipe. Full nutrient profile in link titled 'View full diet information' at instinctpetfood.com/products/raw-longevity-adult-frozen-chicken-patties-dog-food/. Accessed 15 January 2025.

24. Grabska-Kobyłecka I, Szpakowski P, Król A, *et al.* Polyphenols and their impact on the prevention of neurodegenerative diseases and development. *Nutrients,* 2023; 15: 3454.

25. Sechi S, Di Cerbo A, Canello S, *et al.* Effects in dogs with behavioural disorders of a commercial nutraceutical diet on stress and neuroendocrine parameters. *Veterinary Record,* 2017; 80(1): 18.

26. anon. Forza10 Nutraceutic Active Line Behavioral Support Diet dry dog food. https://forza10usa.com/collections/shop-dog/products/behavioral; accessed 02 January, 25.

27. Laterra J, Keep R, Betz LA, *et al.* Blood–brain barrier. In *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects,* 6th edition. Siegel GJ, Agranoff BW, Albers RW, *et al.* (eds). Lippincott-Raven, Philadelphia, 1999.

28. Di Cerbo A, Sechi S, Canello S, *et al.* Behavioral disturbances: an innovative approach to monitor the modulatory effects of a nutraceutical diet. *Journal of Visualized Experiments,* 2017; 119: e54878.

29. Hartwig S, Rankovic A, McCrae P, *et al.* The effects of a plant-based and a plant- and marine-based n-3 oil supplement on behavioral reactivity, heart rate variability, and plasma fatty acid profile in young healthy horses. *Journal of Animal Science,* 2025; skaf117.

30. Hess TM, Rexford JK, Hansen DK, *et al.* Effects of two different dietary sources of long chain omega-3, highly unsaturated fatty acids on incorporation into the plasma, red blood cell, and skeletal muscle in horses. *Journal of Animal Science,* 2012; 90(9): 3023–3031.

31. Whetsell M, Rayburn E. Variation in fatty acids concentration in grasses, legumes, and forbs in the Allegheny Plateau. *Agronomy,* 2022; 12: 1693.

32. Amrit BK, Ponnampalam EN, Macwan S, *et al.* Comprehensive screening and characterization of polyphenol compounds from pasture grasses used for livestock production under temperate region. *Animal Feed Science and Technology,* 2023; 300: 115657.

33. Di Cerbo A, Sechi S, Canello S, *et al.* Adverse food reactions in dogs due to antibiotic residues in pet food: a preliminary study. *Veterinaria Italiana,* 2018; 54(2): 137–146.