

L-tryptophan and alpha-casozepine:

What is the evidence?



Claude Beata
DVM, DECVBM-CA
CETACE Ilc, Toulon, France

Introduction

Since the introduction of a therapeutic food for improving the lives of pets with kidney disease, much attention has focused on the role of nutrition for disease management in companion animals. The benefits of nutritional management for a variety of disorders, including kidney disease, heart disease and allergic dermatitis are well understood. There is now growing interest in the effects of nutrients on behavior in pets, including anxiety and stress-related disorders. While there may not be a consensus on the role of nutrition for managing behavioral disorders, everyone probably agrees that companion animals today frequently experience stressful situations. Helping alleviate stress and anxiety by providing foods that contain specific nutrients with proven anti-anxiety benefits offers a novel approach for behavioral management.

As for other therapeutic options, it is important to consider the available evidence supporting effectiveness of nutritional management or dietary supplements. The best evidence is provided by results of randomized, controlled clinical studies in pets with naturally occurring disease – if a treatment is effective in this situation, it is likely to be effective for your clinical patients. Fortunately, nutritional management with L-tryptophan (L-Trp) and alpha-casozepine (A-Cas) is supported by clinical studies in dogs and cats. This is especially beneficial for cats, where daily administration of treatments can be potentially difficult or even stressful. If food can provide the necessary supplement to decrease anxiety and stress, this makes it easier for the cat and the owner, and likely increases compliance. This article provides an evidence-based review of the effect of dietary ingredients, L-Trp and A-Cas, on modifying anxiety and stress-related behaviors in companion animals, with a primary focus on cats.

Metabolism of tryptophan

Serotonin modulates mood and emotion in animals and human beings; many drugs (eg, fluoxetine) exert their effects by selectively inhibiting reuptake of serotonin at the synaptic cleft. Tryptophan is an amino acid that serves as a precursor for the synthesis of serotonin, one of the major neurotransmitters in



the brain. Serotonin cannot cross the blood-brain-barrier to enter the central nervous system; however, tryptophan and 5-hydroxytryptophan are able to cross via a carrier protein. Tryptophan competes with other large neutral amino acids for transport via this carrier protein; in human beings, it has been shown that increasing the amount of dietary L-Trp (relative to other large neutral amino acids) facilitates transport of more L-Trp into the central nervous system where it is available for serotonin synthesis.^{1,2}

Another forgotten but important component of L-Trp metabolism involves the kynurenine pathway. In most species, 95% of the intake of L-Trp goes into the kynurenine pathway, and only 5% enters the serotonin pathway (Figure 1). This is important because metabolites of these two pathways have different behavioral consequences. This has been extensively studied for decades and the outcome provides useful information related to management of anxiety and stress.³ It has been demonstrated that excitatory neurokynurenines, particularly KYN, have anxiogenic activity in standard animal models, whereas other neurokynurenines have an anxiolytic pharmacological profile.

Another consideration is that species differences exist; it is known that cats have a different enzymatic system⁴ with the major activity of picolinic carboxylase conducting to favor the existence of PICA, and glutamate, respectively an anxiolytic and an excitatory compound (Figure 2).⁵ Two enzymes, tryptophan 2,3-dioxygenase 6 (TDO) and indoleamine 2,3 dioxygenase (IDO), play a major role at the beginning of tryptophan metabolism and appear to be capable of changing the ratio between the serotonin pathway and the kynurenine pathway and are affected by inflammatory states.

Pro-inflammatory cytokines are linked to many behavioral or psychiatric diseases in animals and human beings.⁷⁻¹⁰ They also are known to increase IDO and are capable of increased activity in the kynurenine pathway. Feline stressors (eg, those

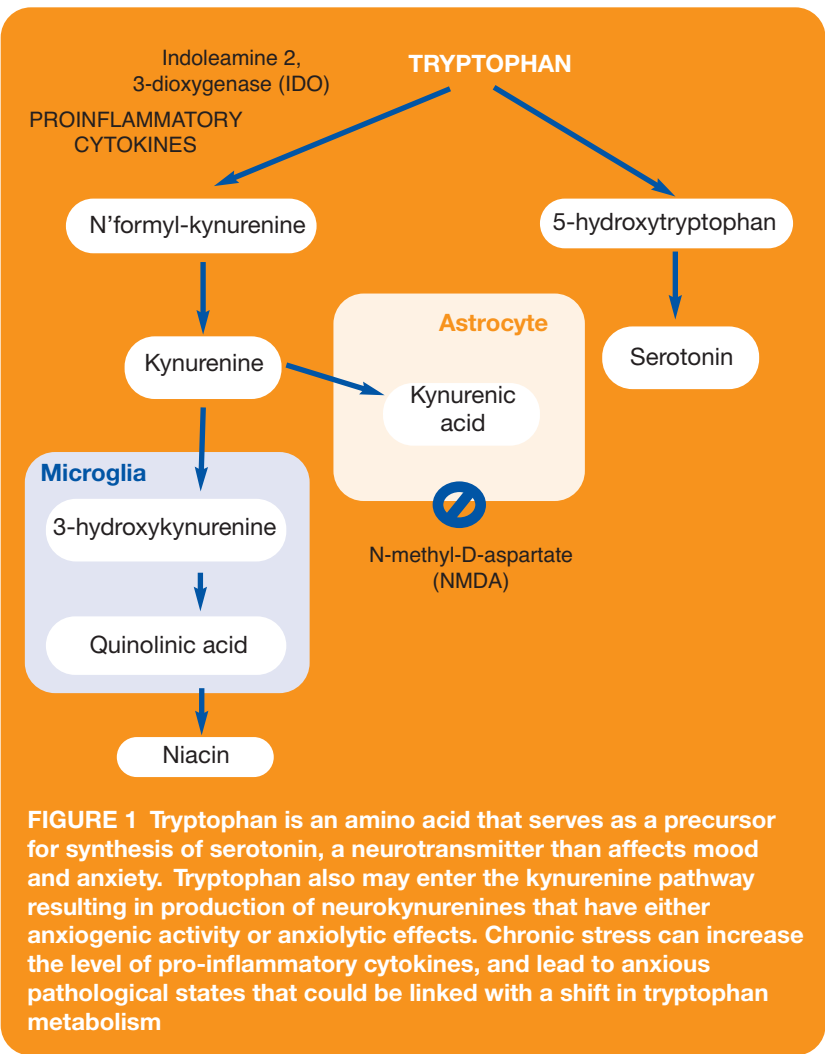


FIGURE 1 Tryptophan is an amino acid that serves as a precursor for synthesis of serotonin, a neurotransmitter that affects mood and anxiety. Tryptophan also may enter the kynurenine pathway resulting in production of neurokynurenines that have either anxiogenic activity or anxiolytic effects. Chronic stress can increase the level of pro-inflammatory cytokines, and lead to anxious pathological states that could be linked with a shift in tryptophan metabolism

associated with unusual events) can increase the level of pro-inflammatory cytokines.¹¹ As a result, chronic stress can lead to anxious pathological states and this could be linked with a shift in tryptophan metabolism. In cats with feline immunodeficiency virus infection, the level of pro-inflammatory cytokines is high; this is one example where increases in metabolism via the kynurenine pathway has been demonstrated.¹²

Effect of L-tryptophan on stress-related disorders

As a precursor of serotonin, L-trp supplementation has been investigated as a possible way of regulating serotonin concentrations in the brain. The use of tryptophan supplementation is based on the fact that low levels of serotonin have been found for some behavioral conditions such as aggression, pointing out the role of this monoamine neurotransmitter. Tryptophan supplementation has been evaluated for its effects in dogs with aggression (dominance or territorial) or hyperactivity.^{13,14} The best results occurred in dogs with territorial aggression when fed a lower protein diet supplemented with L-Trp. The worst results occurred in dogs with dominance aggression when fed a high-protein diet without L-Trp supplementation; however, the effect was not statistically significant.

Studies with L-Trp have also been conducted in dogs and cats to evaluate efficacy for managing stress-related behaviors.^{15,16} Pereira et al presented

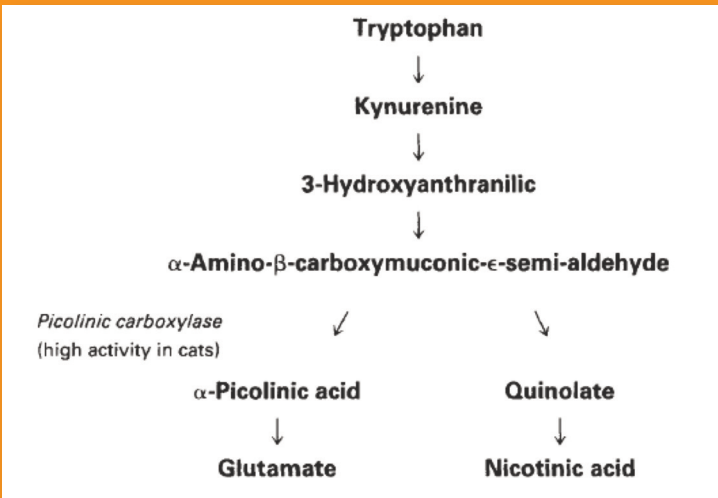


FIGURE 2 Species differences exist regarding metabolism of tryptophan; cats have high activity of picolinic carboxylase, which favors formation of picolinic acid (anxiolytic), and subsequently glutamate (excitatory compound)

findings from two placebo-controlled studies including either working dogs or multi-housed cats. In both studies, there was a 2-week period of habituation, followed by 4 weeks without L-Trp, and then 8 weeks with L-Trp supplementation. In the canine study, there was a statistically significant decrease in barking ($P < 0.05$) and staring ($P < 0.01$). The authors concluded that L-Trp supplementation decreased signs of anxiety and improved animal welfare. In the study of multi-housed cats (a known cause of stress for cats), there were a greater number of statistically significant differences between L-trp supplementation and treatment with placebo (Table 1). The authors concluded that *‘These results suggest that L-Trp supplementation had an effect in changing the frequency of the stress-related behaviors, decreasing anxiety signals. As the L-Trp supplementation reduces some of the cat’s anxiety signs, we conclude that this effect improves their welfare.’* While it is clear that the decrease of some agonistic (fighting) behaviors, house-soiling and scratching are consistent with a decrease of anxiety, the meaning of decreased affiliative, exploring and sustaining behaviors is unclear.

L-Tryptophan appears to be a putative useful option for triggering effects on serotonin-linked behavioral disorders; however, further studies are needed to better understand what happens long-term in stressful conditions. Also, as previously discussed, there are species differences regarding effects of tryptophan and it is important to consider these when evaluating or recommending L-Trp supplementation. For example, in horses, low doses of tryptophan (relative to those contained in commercial preparations) appear to cause mild excitement, whereas high doses reduce endurance capacity.¹⁷ In cats, today, too few things are known about the specificities of tryptophan metabolism, including the possible shift to the kynurenine pathway to be sure of the efficacy.

Effect of alpha-casozepine on anxiety

Cow’s milk has long been considered a beverage with natural ‘tranquilizing’ properties and previous research has confirmed an impact of peptides present in milk.¹⁸ This overall calming effect was observed in babies, and researchers hypothesized that a natural component in the milk was created via digestion (tryptic hydrolysis) by the gastrointestinal tract of babies. Trypsin is more active in the digestive tract of infants whereas pepsin is primarily responsible for protein digestion in adults. α -S₁ casein is one of the major proteins in cows’ milk and studies have demonstrated that peptides derived from this milk protein have strong biological effects.¹⁹⁻²¹ Researchers from a dairy company first identified a decapeptide, obtained via tryptic hydrolysis, whose spatial structure was analyzed and found to be responsible for the anxiolytic activity.^{22,23}

Initial studies in multiple species

As for many other compounds, A-Cas was first tested in classic studies in rats to confirm anxiolytic activity. The conditioned defensive burying paradigm is one of the most classical tests to screen drugs for their potential anxiolytic activity.

Table 1 Signs affected by L-tryptophan supplementation for multi-housed cats (Pereira¹⁵)

Signs (All decreased)	P value
Stereotypies	<0.01
Vocalization	>0.05
Agonistic behaviors	<0.01
Affiliative behaviors	<0.01
Exploring	<0.01
Sustaining behavior	<0.01
House soiling, scratching, agonistic interactions within the group	<0.05

Rats are known to bury aversive stimuli and this has been confirmed as an indicator of anxiety.²⁴ Administration of anxiolytic agents prevents or decreases this conditioned defensive burying response in rats. This test is sensitive because it allows discrimination between anxiolytic effects and effects on general activity. Alpha-casozepine has shown as much efficacy against anxiety as the gold-standard reference molecule, diazepam. Interestingly, A-Cas has not been associated with increased aggression nor loss of working memory, both side effects that may occur with benzodiazepines.²⁵ Similar results have also been found with the elevated-plus maze paradigm, another standard test to evaluate putative anxiolytic effects of drugs.²⁶ Human volunteers have been tested²⁷⁻²⁹ with positive results and two trials have been successfully conducted in dogs, one of which has been published in an international peer-reviewed journal.³⁰

Evaluation of alpha-casozepine in cats

One multi-center, randomized, double-blinded, placebo-controlled clinical trial has been conducted in Europe to evaluate the anxiolytic effects of A-Cas in cats.³¹ Cats were selected from general or specialist practices and investigators had to confirm that cats were not subject to inadequate living conditions. A scale validated by the investigators (all veterinary behaviorist surgeons) was used to evaluate cats included in the study (Table 2).

A total of 34 cats were randomly assigned to receive either A-Cas (15 mg/kg) or placebo once daily for 56 days. Practitioners were allowed to implement classical behavior modifications. Three items were evaluated:

- Overall score.
 - The number of items quoted 0.
 - The owner’s evaluation of the improvement.
- To be assessed as a success, three requirements were needed:
- Overall score equal or superior to 16.
 - Number of items quoted 0 had to equal 0.
 - Owner’s evaluation mark equal or superior to 6 (out of 10).

Of all cats evaluated, 14 were judged to have a successful outcome and 10 of these were in the A-Cas group; this was a statistically significant difference (Chi-square test, 1 df, $P = 0.02$) (Table 3).

Table 2 Scale used to evaluate anxiety in cats at baseline and during/after treatment with either alpha-casozepine or placebo for 56 days

	Contact tolerance with familiars
0	Can't be touched
1	Only short contacts and when the cat initiates it
2	Does not stand by long, provoked or spontaneous, contacts
3	Variable acceptance and seeking of contacts
4	The cat regularly seeks for and accepts contacts – seldom refuses
5	Easily manipulated
	Contact tolerance with non-familiars
0	Systematically disappears or is aggressive
1	Comes to observe but can't be touched
2	Initiates contact after a while but does not accept to be touched
3	Initiates contact after a while and accepts to be touched
4	Accepts non familiars excepts precise categories or individuals
5	Tolerant, friendly and playful with non-familiar as with familiars
	Aggression
0	Hurtful aggression with familiar and non-familiar
1	Hurtful aggression with familiar and non-familiar except one person
2	Possible but infrequent hurtful aggression
3	Threats – without fleeing
4	Threats and flees
5	Never aggressive
	Other fears
0	Frightened by the littlest noise or any new stimulus
1	Difficult exploration, numerous frightening stimuli
2	Fearful but explores after a while
3	Frightened by some precise stimuli
4	Seldom frightened – Calms down quickly
5	Never afraid
	Organic signs
0	Systematic stress-related autonomic signs (\pm displacement activities)
1	Frequent stress-related autonomic signs (\pm displacement activities)
2	Displacement activities with severe consequences (\pm autonomic signs)
3	Displacement activities with medium consequences (\pm autonomic signs)
4	Displacement activities with mild consequences (without autonomic signs)
5	Nothing

Detailed results are summarized in Table 4. This study provides evidence for the efficacy of this product in the management of anxiety in cats, including those in socially stressful conditions. These results confirm the clinical impression of investigators based on testimonials from customers.

Nutritional management of stress

Stress is a normal part of life; however, it may contribute to anxious behavior and it appears to play a key role in the pathogenesis of some diseases such as feline idiopathic cystitis (FIC). Methods to decrease anxiety and stress (eg, environmental enrichment, pheromones, dietary supplements, therapeutic foods with ingredients to help manage stress) are an important component

Table 3 Summary of treatment outcome for 34 cats that received either alpha-casozepine (n = 17) or placebo (n = 17) for 56 days to manage anxiety

	Alpha-Casozepine	Placebo
Successes	10	3
Failures	7	13

Table 4 Summary of results on different items for 34 cats that received either alpha-casozepine (n = 17) or placebo (n = 17) for 56 days to manage anxiety

	Score	Number 0	Evaluation	Familiar humans	Non-familiar humans	Fears	Autonomic signs	Aggressions
Mann Whitney U test	<0.01**	0.04*	0.09 t	0.04*	0.03*	0.09 t	0.16 NS	0.70 NS

t = trend; NS = not significant

of multimodal management for cats with FIC. Use of dietary supplements or therapeutic foods may be helpful; however, it is important to keep some key points in mind:

- The amount of nutrient/ingredient provided on a daily basis should be similar to the minimal requirements that have been established.
- Interactions between the different nutrients/ingredients in the diet need to be studied to determine if there are synergistic, or at least neutral, effects.
- The way the food is provided to the patient may be of major importance because ingredients or nutrients need to achieve a certain threshold to exert a beneficial effect. For example, if food is provided ad libitum and the pet eats multiple small meals throughout the day, as many cats do, is it possible to achieve a therapeutic threshold?

Answers to all these questions require clinical studies with the final product showing that the expected efficacy is present.

Conclusions

Behavioral disorders and stress-related medical conditions can greatly affect the lives of pets today, especially cats. Diets can be useful for managing these situations, and are particularly helpful for cats, a species that may experience additional stress associated with daily administration of any treatment. We continue to learn more about the efficacy of different nutrients and dietary ingredients, and this provides veterinarians with an additional approach to manage anxiety and stress-related behaviors in cats.

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