

# Meal composition and Tryptophan/Large Neutral Amino Acids plasmatic ratio in dog

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*Abstract:* The aim of this study was to assess the impact of meal composition on the plasmatic ratio between Tryptophan (Trp) and 5 other large neutral amino acids (5LNAA: isoleucine, leucine, valine, tyrosine, phenylalanine).

This study included 5 female Labrador Retrievers. Each dog received a meal of puffed rice, minced meat and olive oil (M1) for a single day and then they received a meal of puffed rice and olive oil (M2), with no meat, for another single day. A second meal was administered in the evening to balance the energy intake and both diets were isoenergetic. Blood was collected right before the meal (t0) and after 2, 4, 6, 8 and 10 hours. Trp and phenylalanine concentrations showed no significant difference between M1 and M2 samples. Isoleucine, leucine, valine and tyrosine plasma concentrations were lower after M2. Due to the latter, Trp/5LNAA ratio was higher after the meal with no meat (M2) at all sampling times (except t0) and such trend reached a statistically significant difference at 2 (median: 0.206 versus 0.311), 4 (median: 0.217 versus 0.345) and 10 (median: 0.242 versus 0.289) hours after the meal.

These findings suggest that meal composition has a key role in the Trp bioavailability.

*Key Words:* Tryptophan, LNAA, Serotonin, Behaviour, Meal, Dog, HPLC.

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## Introduction

The relationship between dog and human being is, sometime, negatively influenced by the onset of behavioral problems that may force the owner to relinquish the dog to a kennel (Patronek et al., 1996). Phobias, intra and interspecific aggression and separation problems are frequent behavioral causes of relinquishment (Patronek et al., 1996). These problems can result from many causes, including an altered functionality of serotonergic pathways in the brain, characterized by a serotonin (5-HT) deficiency (Sachs et al., 2015; Rosado et al., 2011).

Tryptophan (Trp) is involved in the synthesis of serotonin (5-HT), melatonin and niacin (Figure 1) but it competes with the other large neutral amino acids for uptake into the brain (Fernstrom, 2013).

Diet manipulation can therefore hypothetically be useful to produce a rise in plasma and, consequently, intracerebral Trp concentrations, leading to the synthesis and release of a greater amount of 5-HT, with positive effects on behavioral problems resulting from an altered function of serotonergic systems.

The aim of this study was to assess the impact of meal composition on the plasmatic ratio between Trp and 5 other large neutral amino acids (5LNAA: isoleucine, leucine, valine, tyrosine, phenylalanine).

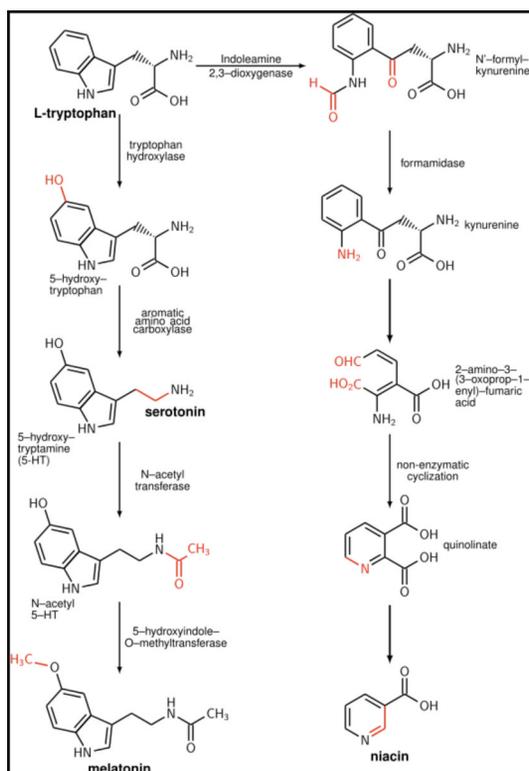


Figure 1. Trp metabolism.

## Materials and Methods

This study included 5 female Labrador Retrievers (2 spayed,  $8.6 \pm 3.8$  years old) from the same bloodline and usually fed the same commercial food once a day. Each dog received a meal of puffed rice, minced meat and olive oil (M1) for a single day and then, after a period of 30 days, during which the dogs returned to their usual diet, they received a meal of puffed rice and olive oil (M2), with no meat, for another single day. A second meal was administered in the evening to balance the energy intake and both diets were isoenergetic. Blood was collected right before the meal ( $t_0$ ) and after 2, 4, 6, 8 and 10 hours. Plasma samples were used for HPLC quantification of Trp and 5LNAA (Wu & Meininger, 2008). At each sampling time, their levels and ratio after M1 and M2 were compared using Wilcoxon rank-sum test ( $p < 0.05$ ).

## Results

Trp and phenylalanine concentrations showed no significant difference between M1 and M2 samples. Isoleucine, leucine, valine and tyrosine plasma concentrations were lower after M2. Due to the latter, Trp/5LNAA ratio was higher after the meal with no meat (M2) at all sampling times (except  $t_0$ ) and such trend reached a statistically significant difference at 2 (median: 0.206 versus 0.311), 4 (median: 0.217 versus 0.345) and 10 (median: 0.242 versus 0.289) hours after the meal (Table 1).

Table 1. AA concentrations in the two different diets; median values with min-max range in parenthesis; different superscript letters show statistically significant differences between M1 and M2.

Time	ILE (µg/ml)		LEU (µg/ml)		PHE (µg/ml)		TYR (µg/ml)		VAL(µg/ml)		TRP(µg/ml)	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
00 h	<b>8.3</b>	<b>7.6</b>	<b>17.0</b>	<b>16.8</b>	<b>11.4</b>	<b>10.0</b>	<b>12.3</b>	<b>12.5</b>	<b>25.1</b>	<b>24.9</b>	<b>16.3</b>	<b>15.1</b>
	(7.1-9.9)	(6.5-9.5)	(14.7-20.8)	(13.4-21.4)	(9.5-14.2)	(9.9-15.9)	(10.3-14.9)	(10.0-13.7)	(24.2-30.3)	(21.8-29.6)	(13.7-17.8)	(14.5-20.7)
02 h	<b>10.9<sup>a</sup></b>	<b>5.3<sup>b</sup></b>	<b>20.1<sup>a</sup></b>	<b>10.9<sup>b</sup></b>	<b>12.0</b>	<b>8.3</b>	<b>14.5<sup>a</sup></b>	<b>9.9<sup>b</sup></b>	<b>29.9<sup>a</sup></b>	<b>18.4<sup>b</sup></b>	<b>19.4</b>	<b>16.3</b>
	(9.3-12.7)	(4.0-5.6)	(17.4-21.9)	(7.9-12.8)	(9.6-14.6)	(6.9-13.0)	(11.6-18.6)	(6.5-10.8)	(25.4-31.1)	(16.2-22.4)	(16.9-22.1)	(13.00-19.9)
04 h	<b>10.4<sup>a</sup></b>	<b>4.7<sup>b</sup></b>	<b>18.8<sup>a</sup></b>	<b>10.3<sup>b</sup></b>	<b>10.0</b>	<b>8.9</b>	<b>11.9<sup>a</sup></b>	<b>10.0<sup>b</sup></b>	<b>27.9<sup>a</sup></b>	<b>15.9<sup>b</sup></b>	<b>17.5</b>	<b>15.3</b>
	(9.0-11.9)	(3.0-5.3)	(17.6-19.3)	(6.9-12.7)	(9.1-13.5)	(6.6-11.4)	(10.8-13.7)	(6.0-11.5)	(26.0-30.0)	(13.5-19.4)	(16.5-20.1)	(12.6-20.2)
06 h	<b>8.3<sup>a</sup></b>	<b>5.0<sup>b</sup></b>	<b>15.1<sup>a</sup></b>	<b>10.9<sup>b</sup></b>	<b>8.5</b>	<b>9.0</b>	<b>9.6</b>	<b>10.8</b>	<b>23.1<sup>a</sup></b>	<b>17.1<sup>b</sup></b>	<b>16.4</b>	<b>15.5</b>
	(6.9-9.9)	(4.3-5.6)	(13.9-16.5)	(8.9-12.0)	(8.0-11.9)	(7.4-13.3)	(8.3-10.2)	(7.4-11.8)	(21.4-26.3)	(15.3-19.2)	(13.7-18.8)	(13.1-21.4)
08 h	<b>7.8<sup>a</sup></b>	<b>5.3<sup>b</sup></b>	<b>14.9<sup>a</sup></b>	<b>11.6<sup>b</sup></b>	<b>9.9</b>	<b>9.4</b>	<b>10.0</b>	<b>11.3</b>	<b>22.1<sup>a</sup></b>	<b>17.2<sup>b</sup></b>	<b>14.5</b>	<b>14.2</b>
	(6.7-10.5)	(4.6-6.2)	(12.8-18.8)	(11.0-12.9)	(8.3-12.2)	(8.3-12.5)	(7.5-13.4)	(7.7-11.5)	(19.7-25.9)	(15.5-18.4)	(13.7-20.3)	(13.0-20.4)
10 h	<b>7.9</b>	<b>6.2</b>	<b>15.2</b>	<b>12.4</b>	<b>11.4</b>	<b>11.9</b>	<b>11.2</b>	<b>13.5</b>	<b>20.4<sup>a</sup></b>	<b>17.3<sup>b</sup></b>	<b>16.0</b>	<b>17.5</b>
	(6.7-10.6)	(4.3-7.9)	(13.4-19.6)	(10.8-16.9)	(9.2-12.4)	(10.9-13.3)	(9.3-13.9)	(10.5-13.9)	(18.2-27.7)	(15.7-20.9)	(13.5-24.0)	(15.9-22.8)

## Discussion

To our knowledge, the results of this preliminary study are the first data about the bioavailability of the plasma Trp in the dog after two daily meals with different percentages of proteins and carbohydrates. Data suggest that M2 can significantly modify the Trp/5LNAA's ratio, keeping it higher than M1. This finding might have a strong impact, because the synthesis and release of 5-HT by brain neurons is rapidly influenced by the local Trp concentration (Sharp, 1992).

An increase in Trp levels in plasma and, consequently, of 5-HT in brain could have positive effects on some dog behavioral problems, characterized by a deficit of this neurotransmitter.

However, the adoption of a dissociate diet, although probably easily accepted by the owners, requires some caveats due to hyperglycemic peak.

## Conclusion

These findings suggest that meal composition has a key role in the Trp bioavailability.

However, further studies, observing behavioral changes and assessing serotonin and melatonin levels, are required to evaluate the impact of Trp/LNAA's ratio on dog behavior. In addition, further research should clarify how to deal with possible side effects of a dissociated diet, such as the potential hyperglycemia after the carbohydrates meal.

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### Composizione del pasto e rapporto plasmatico di Triptofano/grandi aminoacidi neutri nel cane

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#### Sintesi

Lo scopo dello studio è stato quello di valutare l'impatto della composizione del pasto sul rapporto plasmatico di Triptofano (Trp) e 5 altri aminoacidi neutri (5LNAA: isoleucina, leucina, valina, tirosina e fenilalanina).

Nello studio sono state incluse 5 femmine di Labrador Retriever. Ogni cane ha ricevuto un pasto costituito da riso soffiato, carne tritata ed olio di oliva (M1) per un solo giorno e quindi, dopo 30 giorni di dieta normale, un pasto costituito da riso soffiato ed olio di oliva (M2), senza carne, per un altro solo giorno. Un secondo pasto era somministrato la sera per bilanciare l'apporto energetico. Entrambe le diete (M1 e M2) erano isoenergetiche.

Il sangue era prelevato appena prima del pasto (t0) e dopo 2,4,6,8, e 10 ore. Le concentrazioni di Trp e fenilalanina non mostrarono differenze significative tra M1 e M2.

Le concentrazioni di isoleucina, leucina, valina e tirosina nel plasma erano più basse dopo M2. Per questo motivo il rapporto Trp/5LNAA fu più alto dopo il pranzo senza carne (M2) a tutti i tempi campionati (eccetto t0) e questo trend raggiunse una differenza statisticamente significativa a 2 (mediana: 0,206 versus 0,311), 4 (mediana: 0,217 versus 0,345) and 10 (mediana: 0,242 versus 0,289) ore dopo il pasto.

Questi risultati suggeriscono che la composizione del pasto ha un ruolo chiave nella biodisponibilità del Trp.