

PLASMATIC TRYPTOPHAN/LARGE NEUTRAL AMINO ACIDS RATIO IN DOMESTIC DOGS IS AFFECTED BY MEAL COMPOSITION

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Tryptophan (Trp) is involved in the synthesis of serotonin and melatonin and it competes with other large neutral amino acids (LNAAs) for its uptake into the brain [1]. The aim of this study was to assess the impact of three different diets on plasmatic Trp/LNAAs ratio. This study included five female Labrador Retrievers (2 spayed, 8.6 ± 3.8 years old) from the same bloodline, who were usually fed the same commercial dry food once a day. Each dog received three different diets for one single day each. Isocaloric and isonitrogenous diets, with a carbohydrates content of 47% and proteins content of 28% on dry matter basis, were provided in two meals, one in the morning and one after 12 hours. Dogs received the first diet (D1) and then they returned to their normal diet for 30 days. After that “washout” period, dogs were fed with the second diet (D2), and after 30 more days they received the third diet (D3). D1 was composed of a mix of puffed rice, minced meat and olive oil equally divided into the two meals. D2 was made up of two different meals. The morning meal was composed of puffed rice and olive oil, whereas the evening meal consisted of minced meat and olive oil. D3 consisted of two identical meals of the commercial dry food usually consumed by the sample dogs. Blood was collected right before the first meal (t0) and after 2, 4, 6, 8, 10 and 24 hours. Plasma samples were used for HPLC quantification of Trp and other LNAAs (isoleucine + leucine + phenylalanine + tyrosine + valine) using a method described in literature [2]. Their levels and ratios at t0 and after D1, D2 and D3 were compared using a mixed model for repeated measures ($p < 0.05$). Trp concentrations showed no significant difference between D1, D2 and D3 samples at any sampling times. LNAAs levels were similar at t0 in the three experimental days, but they showed different trends depending on the composition of the meal provided. In particular, D2 led to a decrease in LNAAs levels and therefore to higher Trp/LNAAs ratios in the 6 hours period after the provision of carbohydrates. In detail, mean Trp/LNAAs ratio of D2 was statistically higher compared to both D1 and D3 at t2 (D1=0.224; D2=0.306; D3=0.217; $p < 0.001$), t4 (D1=0.225; D2=0.327; D3=0.197; $p < 0.001$), and t6 (D1=0.244; D2=0.303; D3=0.205; $p < 0.015$). In addition, mean Trp/LNAAs ratio after D2 was higher than after D3 also at t8 (D2=0.280; D3=0.206; $p < 0.001$) and t10 (D2=0.294; D3=0.224; $p < 0.001$). The trend was different at t24, when Trp/LNAAs ratio was found to be significantly lower after being fed D2 compared to D1 (D1=0.210; D2=0.155; $p = 0.041$). These results indicate that the diet affects Trp bioavailability. Therefore, it is worthwhile to investigate the effects of diet on Trp bioavailability at the brain level, serotonin and melatonin secretion and the real impact of Trp/LNAAs ratio on dog behaviour.

[1] Fernstrom JD. Large neutral amino acids: dietary effects on brain neurochemistry and function, *Amino Acids*, 45:419–430, 2013. [2] Wu G. Determination of proline by reversed-phase high-performance liquid chromatography with automated pre-column o-phthalaldehyde derivatization, *Journal of Chromatography*, 641:168-175, 1993.