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Original Research

Developmental Changes During the First Year of Life in Plasma Tryptophan Concentration of the Foal

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ABSTRACT

Circulating tryptophan (TRP) levels can influence protein synthesis in various tissues during early stages of life. It is important to investigate factors that influence TRP dynamics to evaluate results of TRP use in equines. Previous studies have demonstrated that plasma TRP levels in the first periods of life are indicative of brain serotonin synthesis and that in horses there may be breed differences in the behavioral response to TRP administration. Current research has investigated the pattern of plasma TRP concentration in growing foals of two different breeds: Anglo-Arabian (AA) and Sicilian Oriental Purebred (PSO). Blood samples were collected from 10 clinically healthy colts, ages 1 to 12 months old. Plasma TRP concentrations were determined by high-performance liquid chromatography. Two-way analysis of variance showed age- and breed-related differences. A positive significant correlation was found between age and TRP concentration ($r = 0.6$, $P < .001$). After weaning (7th month of age) TRP levels were significantly higher than in previous months. PSO colt foals had TRP levels significantly higher than AA colt foals in the 1st, 2nd, and 12th months of life. These findings suggest that plasma TRP dynamics in growing horses are influenced by age and breed and should be taken into consideration for TRP experiments in equines.

1. Introduction

L-Tryptophan (TRP) is an essential amino acid that may affect many important biological processes in animals and in humans. The metabolism of TRP is complex and influenced by different factors; in fact, many studies of the effects of TRP administration have been conducted in horses [1,2], but changes in plasma TRP concentration according to several physiological variables are still not clear. TRP is a precursor of serotonin, and an increase in this neurotransmitter in the brain has been associated with sleepiness, sedation, and suppression of aggression [3]. TRP supplements have been used by horse owners as calmative for excitable horses [4] and to reduce cribbing behavior [5]. Therefore, the results of the few studies that focused on behavioral effect of TRP administration in horses showed that TRP administration caused mild excitation [6] and not behavioral changes [7]. It is important to investigate factors affecting TRP metabolism to be able to evaluate critically the results of TRP experiments. Excitability is a component that in horses has shown the highest level of variability among breeds [8], and some reports suggest there may be breed differences in behavioral response to TRP administration [6]. Levels of circulating TRP are higher in mares than in geldings [9] and higher after food ingestion [10] and show nycthemeral change [11]. Studies conducted with athletic horses suggest that exercise influences TRP levels depending on different workloads [12-14]. Plasma amino acids related to age in foals have been widely studied during the

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first weeks of life [15-17], but reports of their modification during the first year of life have been few [17]. Reports in humans indicate that plasma TRP is affected by age in adults and in the pediatric population [18,19]. The aim of this study was to evaluate the influences of age, breed, and weaning on plasma TRP modifications in 10 foals.

2. Material and Methods

Five Anglo-Arabian (AA) and five Sicilian Oriental Purebred (PSO) colt foals belonging to an equine farm in Sicily (latitude 37°17'43"N, longitude 14°50'34"E) were used in this study. The foals were born full term in March 2011. Before the start of the study, all subjects underwent a heart examination, respiratory auscultations, and routine hematology and plasma biochemistry testing. Only clinically healthy animals were used. Foals stayed with their mothers from birth until weaning at 6 months. Body weight was predicted by body measurements [20] at 6 and 12 months of age. The mean body weight of AA foals was approximately 230 ± 6 kg at 6 months of age and 340 ± 11 kg at 12 months of age. The mean body weight of PSO foals was approximately 185 ± 7 kg at 6 month of age and 270 ± 9 kg at 12 months of age. Foals (sucklings and weanlings) were housed on pasture with free access to grass and lucerne hay (digestible energy, 8.6 MJ/kg; 18.2% crude protein) and from the 3rd month of life received twice-daily supplementation (1 kg/100 kg of body weight) of a commercial creep feed fortified with vitamins and minerals, which ensured the recommended percentage of total protein (18%), crude fiber (10.30%), calcium (0.9%), and phosphorus (0.6%), and they received water ad libitum. Daily supplementation of creep food was supplied in feeders, which mares could not access. The amino acid composition of creep food (% of dry matter) is shown in Table 1. All housing and care conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/ EU. All blood samples were collected from each foal by the same operator every 30 days from 1st to the 12th month of life by jugular venipuncture, using vacuum tubes with heparin, between 9:00 and 10:00 AM in order to avoid influence of daily variation observed for TRP in horses [11]. The sample taken at the 7th month of life was obtained 2 week after weaning. When foals were younger than 7 months, no attempt was made to control the time of withdrawal of blood samples in relation to feeding time, as they were still nursing.

The tubes were centrifuged (2,500 × *g* for 10 minutes) at 4°C to obtain plasma that was kept at −20C° and analyzed within 1 month. An isocratic high-performance liquid chromatography (model 1525 binary HPLC pump; Waters, Milford, MA, USA) method was used for the separation and quantification of plasma tryptophan. The mobile phase used was a water solution of 0.0493 M Na_2HPO_4 and 0.0382 M citric acid (pH, 3.8)-methanol (85:15, v/v). Twenty microliters of supernatant was injected into a C_{18} octadecylsilane column (250 mm × 4.6 mm; Waters). The flow rate was 1.2 mL/min. The detection system was an electrochemical detector (model 5100A; Coulochem ESA).

Distribution of data was analyzed by Kolmogorov-Smirnov test. Data were normally distributed, and the influences of age and breed were analyzed by two-way analysis of variance (ANOVA). Post hoc differences of age were identified using the Bonferroni multiple comparison test with a significance value set at $P < .05$. The effect of two breeds was also tested by unpaired *t*-test at each month. Data were analyzed using Statistica version 7.0 software (Stat. soft. Inc. Tulsa, OK).

Table 1
Amino acid composition of creep feed for growing foals

Amino Acid	% of Dry Matter
Lysine	0.63
Methionine	0.27
Threonine	0.53
Arginine	0.92
Leucine	1.0
Isoleucine	0.55
Valine	0.73
Histidine	0.38
Phenylalanine	0.63
Tryptophan	0.18

3. Results

Table 2 shows the means ± standard deviations (SD) of TRP concentrations in foals, from the 1st to the 12th month of life. A significant effect of age was found ($F_{11,99} = 9.68$, $P < .001$). Significance levels for each age month are also shown. The highest levels were found when foals were 12 months old: 97.66 ± 10.32 μmol/L, the lowest levels when they were 4 months old: 42.38 ± 15.15 μmol/L. A significant and positive correlation was found between age and tryptophan levels in total ($r = 0.60$, $P < .001$) and for PSO ($r = 0.54$, $P < .001$) and AA ($r = 0.74$, $P < .001$) foals. The influence of breed was significant ($F_{1,99} = 13.18$ $P < .01$). PSO colt foals showed TRP levels significantly higher ($P < .01$) than those of AA colt foals during the 1st, 2nd, and 12th months of life.

4. Discussion

The results obtained provide preliminary evidence that TRP plasma levels in growing horses are influenced by age and by breed. TRP plasma concentrations significantly increased from the 7th to the 12th month of life; the highest values were found in yearlings (12 months of life) compared to previous months. Overall, the concentrations of TRP in plasma of foals were very similar to those reported by other studies [17] in Standardbred foals during the first 3 months of life, although levels in our study were higher than those reported by the same studies at 6, 9, and 12 months of age. The reason for this difference is unclear, but diet, environmental conditions, and breed could influence these results in weanling and yearling horses. In the pediatric population, plasma TRP and some other amino acids showed a steady increase throughout infancy, childhood, and adolescence [19], and levels were lower in older than in the younger subjects [18]. Some studies suggest that food deprivation in foals [16] and feed intake in yearling horses [10] could influence TRP levels. It is unlikely that the increase in TRP levels from the 7th month of age could be the effect of changing milk to hay because from 8 weeks of life onward the foals consume higher amounts of fodder than milk. In