Plasma serotonin in horses: comparison between two different management conditions

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Recent reports of new and important roles for serotonin in the periphery have served to increase interest in circulating serotonin (5-HT). The much smaller pool of free (extraplatelet) plasma 5- HT is accessible to sites of action and receptors, and may be important in many processes. Assessing this extraplatelet plasma pool could be very difficult also because many factors could influence 5-HT levels.[1] Horses kept in stalls are deprived of opportunities for social interactions and the performance of natural behaviors is limited. The hypothesis of this study was that stalling horses results in a negative effect on their welfare. As marker of poor welfare we evaluate plasma 5-HT and its precursor tryptophan (TRP) in 14 adult horses heterogeneous for sex, breed and age (13±7 years). In previous studies lower levels of plasma 5-HT were found in horses with cribbing behaviour[2] and in subjects feed with high levels in concentrates.[3] Horses of this study were divided in two groups: Stall (S) Group and Pasture (P) Group. Group S (n.6 horses) was maintained in individual box under a natural photoperiod (sunrise at 06:06, sunset at 18:49) and natural indoor temperature (19-21°C) from the day before the experiment to the afternoon of follow day. Horses were fasted overnights (12-14 hours) and then feed with hay that was provided at 08:30 and 12:30, water was available *ad libitum*. Group P (n.8 horses) was maintained at the same condition of Group S until 8:30 then it was transferred from box stalls to pasture. Blood samples were obtained from the jugular vein at 08:00, 12:00 and 16:00 and collected into EDTA-containing tubes. Within 30 minutes from the venipuncture, sample tubes were centrifuged at 1350 x g for 10 minutes to obtain the fraction defined as platelet poor plasma. One hundred µL of plasma were then supplemented with an equal volume of an internal standard represented by N-methylserotonin and treated with 100 µL of a precipitating reagent to ensure protein removal. Samples were the vortex-mixed for 30 seconds, allow to stand for 10 minutes at 4°C and centrifuged in a top-bench centrifuge at the maximal speed. The resulting clear supernatants were stored at -20°C and analysed within one week for the HPLC quantification of 5-HT and TRP according to protocols earlier described.[4] All the results obtained were expressed as mean values±standard deviation (SD). One-way repeated measure analysis of variance (ANOVA) was performed to determine the statistically significance and Bonferroni's test was applied as post hoc comparison test. Mann Whitney test was used to compare differences between groups. The data were analysed using the software STATISTICA 8 (Stat Soft Inc.). Results for 5-HT and TRP are shown on Table 1 and Figure 1 respectively. The influence of time evaluate by ANOVA was significant in both Groups ($P<0.001$) with levels significantly higher at 12:00 and at 16:00 compared to levels at 08:00. 5-HT levels were significantly higher in Group P compared to Group S at 12:00 ($P<0.01$) and 16:00 ($P<0.001$). Also TRP levels were significantly influenced by time in both Groups ($P<0.001$) with higher levels at 12:00 and at 16:00 compared to levels at 08:00. No difference between Groups were found for TRP concentrations. The pattern of 5-HT and TRP levels confirmed previous results on equine daily rhythms for these parameters.[5] The lower levels of 5-HT measured at 12:00 and at 16:00 in Group S could indicate that factors as absence of exercise and isolation could influence 5-HT levels. Regardless of this, we recognize that, in addition to differences in exercise and social interaction there are confounding factors between treatment groups including nutrition rate and exposure to sunlight. However, these same confounding factors would be present in any operation where a decision has to be made as to whether to stall horses or provide access to pasture. In conclusion obtained results in the present study showed the modulation of plasma 5-HT by two different management conditions. Our suggestion is to improve the knowledge about factors that can increase plasma equine 5-HT levels in order to guarantee the animal welfare.

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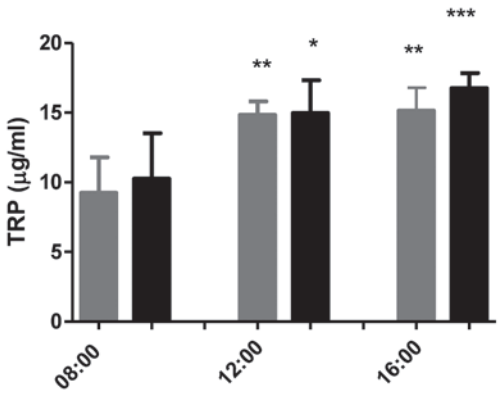

Figure 1. Patterns of mean values (±standard deviation) of plasma tryptophan in Group S (grey bar) and in Group P (dark bar) from 08:00 to 16:00.

Tabella 1. Patterns of mean values (±standard deviation) of plasma 5-HT (ng/mL) from 08:00 to 16:00 in horses maintained in individual box (08:00 both groups, 12:00 and 16:00 Group S) and at pasture (Group P: 12:00 and 16:00).

Time	Experimental condition			ANOVA
	08:00	12:00	16:00	
5-HT ng/mL (Group S)	29.4±10.0	110.9±27.1^{c}	121.7±31.2^{c}	F2,21=33.66; P<0.001
5-HT ng/mL (Group P)	23.0±4.78	42.04±6.8^{b*}	68.13±11.2^{c**}	F2,15=47.81; P<0.001

Bonferroni post-hoc comparison: *vs* 08:00 ^{c}P<0.001; ^{b}P<0.01. Mann-Whitney test: *vs* Group S * P<0.01; **P<0.001.

References

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