

HORSES, BUT NOT MULES, ARE STRESSED WHEN APPLYING A SIGNIFICANT EFFORT TO REACH FOOD

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Aiming to better improve animals conditions and based on the Dawkin's (2006) proposal to search for what animals want instead of just looking for welfare indicators, many preference tests have been applied to determine such animals wants. However, as suggested by Duncan (2006), it also seems relevant to determine the importance of the environmental resources for the animals. In this context, many authors have tested how much animals will pay to access specific resources. Such an approach has usually been evaluated by exposing the animals to situations in which they need to spend physical effort by pushing weighed doors or repeating pre-conditioned or even natural behaviors to access a specific resource. As it is likely that animals spending physical effort are stressed, the intensity of stress responses may be associated with the intensity of effort applied to access a relevant resource. However, to our knowledge, there is no study evaluating such association of these responses. Taking into account that food is considered one of the resources that the animals are more motivated to reach (Mason et al., 1998), here we tested if the stress response of horses and mules can indicate the effort expended to access food. More specifically, we evaluated if the levels of cortisol hormone (one of the most reliable variables that indicate stress response) of horses (n= 8) and mules (n= 8) increase proportionally with the raise of physical effort required to access food. Thus, the access to concentrated food (commercial ration) was blocked by a portress, whose weight was gradually increased over three days of testing. On the first day, it was not added any weight to the portress; in the second day, we added 200% of the initial weight of the portress; and finally, in the last day, the portress was completely locked. This latter test was done in order to obtain the animals response in case of complete inability to pass through the portress, which enabled us to determine the stress response when the animals need to pay the maximum price to access food. The testing order of individuals in each day was randomized. In each test, we collected blood samples of each individual immediately before the effort tests (pre-effort cortisol) and also 30 min after the effort tests had started (post-effort cortisol), based on that 30 min after a stress situation may represent a peak response of cortisol for horses (Petters et al. 2011). In cases of animals passing through the portress before completing the 30 min period, they did not receive the concentrated food immediately, but only when this period of time was concluded. This was done to avoid possible influence of reward effects over stress response. From these blood samples, we determined the concentrations of plasma cortisol by radioimmunoessay technique. We found a significant decrease for horses (repeated measures ANOVA, $P < 0.05$) and a

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strong tendency to decrease for mules (repeated measures ANOVA, $P=0.063$) of pre-effort cortisol concentrations on the last day of testing. When comparing the variation of cortisol levels (pre-effort subtracted from post-effort levels) among the three days of test, in the last day (locked portress) horses had an increase in the cortisol levels after the effort test, indicated by a positive value, in contrast with the responses observed in the other two days of test. Such difference was significant (repeated measures ANOVA, $P<0.05$). However, mules did not express any significant difference among the three days of test, being that the cortisol variations were always negative. Although the pre-effort cortisol levels seemed to decrease over time for both horses and mules, this was probably a consequence of the habituation of the animals to the test situation and should not explain our results, especially considering the differences reported between both species. Thus, we conclude that horses are stressed when applying a maximum effort to access food, which may represent a frustration response caused by the inability to reach such reward; a response not observed in mules.

Keywords: motivation test; cortisol levels; frustration; concentrated food.

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