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Original Research

Acute Effects of a Single-Dose Nutritional Product on Stress Response and Task Completion in Horses

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ABSTRACT

There is a growing number of nutraceutical products for promoting tractability and reducing anxiety in horses, despite a virtual absence of scientific assessment of these products. The objective of this study was to compare the effects of acepromazine (ACE) and a magnesium-based antianxiety formulation in horses participating in tasks typical of normal equine management. Six horses were randomly allocated to one of the three treatment groups in a 2 × 3 randomized block design. Each horse was fitted with a heart-rate (HR) monitor, and received a single dose of ACE (0.5 cc/horse), the nutritional formulation (NUT), or a placebo paste exactly 30 minutes before commencement of tasks. Tasks included loading onto an equine weighbridge, loading onto a two-horse trailer, unaccompanied transport in a two-horse trailer, jugular venous blood sampling, and 10 minutes in a stall with an unfamiliar object. Stress response with respect to peak and average HR, time to completion of tasks, and plasma cortisol were measured. This study demonstrated that tasks inherent to modern equine management are sufficient to induce a stress response, as demonstrated by increased peak and average HRs, and increased plasma cortisol. The average HR of the earliest task (30 minutes after ACE and NUT administration) was effectively blunted by administration of ACE or NUT 30 minutes before onset of stress. It is concluded that oral ACE and NUT reduce HR_{avg} during a stressful task with which they were challenged 30 minutes after administration. Effects of these treatments on plasma cortisol require further research.

1. Introduction

Anxiety and intractability in horses is a very common complaint among horse owners. Unfortunately, this behavior is frequently anthropomorphized and incorrectly bestows on the horse a uniquely human attribute of being able to rationalize compliance. In reality, the resistant, anxious horse is reacting to the complex physiological environment inherent in every animal of prey. Stress hormones such as adrenaline and cortisol spike in response to environmental challenges, and trigger the “flight or fight” response that horse owners unwittingly invoke on a daily basis. Recently, it has been reported that energy and/or behavioral problems are the most, and third most, important health and performance issues facing high-level dressage and eventing horses, respectively, in the United Kingdom [1]. A lack of research in the area of nutraceutical interventions for equine intractability contributes, at least in part, to poor representation of calming products in horse owner purchasing of nutritional supplements [1].

Among the most common anxiolytic drugs used for horses is acepromazine (ACE). Acepromazine is neuroleptic agent that produces significant reduction in environmental reactivity without concurrent analgesia [2]. However, there are reported adverse effects of ACE in horses, including tachycardia [3], penile prolapse, and hypotension [4]. Furthermore, the drug is a restricted substance for

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competition horses and cannot be used within 144 hours of competition [2]. Thus, it is of great interest to validate the use of a nonrestricted anxiolytic product that produces the desired reduction in nervous tension.

Nutritional approaches to nervous disorders in horses are an appealing, if underinvestigated, option for horse owners [5]. The most recent review of an antianxiety nutritional ingredient (L-tryptophan) in horses [6] drew the vast majority of research from nonequine species. Two papers have since been published which report behavior-modifying effects of an oral casein extract (α-casozepine) [7,8] based on ranking of horses expressing aversive behavior. These studies reported favorable behavioral outcomes of the treatment, but neither included objective physiological outcome measures associated with anxiety. The product under investigation for the present study was comprised of magnesium and thiamine in a guar gum base. Magnesium is a physiological N-methyl-D-aspartate (NMDA) receptor antagonist [8], and has been shown to reduce anxiety [9-11], as well as to adapt the brain to stress without reducing learning capacity [12] . Similarly, supplemental thiamin (vitamin B1) is as effective as exercise in reducing biomarkers of anxiety in rats [13] and, when combined with magnesium and zinc, reduces clinical signs of postpartum depression in mice [14].

The purpose of the present study was to quantify the effect of an oral single-dose nutraceutical paste on physiological and behavioral indicators of stress in horses. It was hypothesized that horses receiving the paste would exhibit a lower stress response, as measured by lower peak and average heart rates (HRs) and plasma cortisol, and perform tasks faster compared with when performing the tasks without the treatment.

1.1. Methods

Use of animals for this study was reviewed and approved by the Nutraceutical Alliance Animal Care Committee, in compliance with the Ontario Animals for Research Act. Owners of all horses participating in the study signed an informed consent. Six horses (Table 1) were selected from a facility in Campbellville, Ontario based on owner-reported aversion to at least one of the following tasks:

Task 1: loading onto and/or standing for 3 seconds on an equine weighbridge
Task 2: loading onto a two-horse trailer

Table 1
Description of horses.

Horse Number	Age, y	Gender	Breed	Body weight, kg
1	6	G	Quarter Horse	472
2	8	M	Irish Sport Horse	386
3	13	M	Thoroughbred	475
4	4	G	Warmblood x Draft	545
5	9	G	Andalusian x Draft	506
6	18	G	Morgan	414
7	3	M	Warmblood X	424
8	5	M	Thoroughbred	495

Task 3: transport alone in a two-horse trailer for a 28-km route (approx. 25 minutes)
Task 4: jugular venous blood sample
Task 5: stall neophobia (horse was walked into a stall containing an unfamiliar object. Object was changed week-to-week to avoid habituation responses)
Week 1: white board with black irregular painted circles, hung from a wall
Week 2: one orange and one blue tarp hung from a wall
Week 3: small opened umbrella and artificial greenery

Horses were kept in a group-housing system with unrestricted access to a bedded free-stall barn and approximately 30 acres of grass pasture. They received supplementary diets according to their nutritional requirements (NRC 2007), which included free choice mixed grass and/or legume hay and trace mineral salt. Horses also received individualized concentrate rations containing 12% forage pellet, unmolassed beet pulp pellets, ground flax meal, and/or a ration balancer as needed (Equilizer; Purina).

Test days occurred every Wednesday and Friday (three horses per day) for 3 weeks in July. Ambient temperature during execution of the tasks was 24°C–32°C. The time of day and sampling order of horses was conserved week to week to control for diurnal variation in plasma cortisol [15].

Horses were assigned to one of the three treatment groups in a randomized block design. Each week each horse received a different test product so that by the end of the study each horse had received each of the three test products, for a final "*n*" of six per treatment group.

1.2. Treatment Groups

- a single oral dose of a paste containing guar gum and water (CON)
- a single oral dose of a paste containing guar gum, water, thiamine, magnesium citrate, and glycerin (nutritional formulation [NUT]; Calm and Collected. Selected Bioproducts Inc, Guelph, Ontario, Canada)
- a single sublingual dose of ACE (0.5 cc)

On test days, all horses received their normal breakfast ration at least 1 hour before commencement of the challenge tests. In sequence, they came into the barn and a jugular venous blood sample was obtained for baseline plasma cortisol. Horses were then fitted with a saddle and HR monitor (Equine Inzone; Polar Canada), and placed into a stall with hay until their HR stabilized to resting level. Heart rate was checked every 2 minutes. When measured, HR was within 2 beats/min, it was considered to be "resting". Once resting, HR was recorded, horses were dosed with their allocated treatment and then returned to the stall for 30 minutes. After 30 minutes, horses were taken from the stall and the HR monitor was set to record peak and average HRs individually for each of the tasks. Tasks were conducted in the same order each time, as described above. The same handler (WP) handled the horses during each task. Time taken to completion of each task was recorded, up to a maximum of 10 minutes. If a