



Original Research

Modestly Improved Compliance and Apparent Comfort of Horses With Aversions to Mildly Aversive Routine Health Care Procedures Following Short-Term Alpha-Casozepine Supplementation



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a r t i c l e i n f o

Article history:

Received 17 March 2014

Received in revised form 20 May 2014

Accepted 5 June 2014

Keywords:

Alpha-casozepine

Horse

Behavior modification

Anxiolytic

a b s t r a c t

Alpha-casozepine, a decapeptide derived from bovine milk α -S₁ casein, has well-documented anxiolytic properties in several species. To evaluate potential benefit of alpha-casozepine to horses' compliance and comfort with routine management and health care procedures, we blindly compared behavior of alpha-casozepine-supplemented and control-supplemented horses with known specific aversions to specific health care procedures. Twenty-six light horse mares were first screened for aversions based on compliance and apparent comfort during a standard battery of 12 health care examinations and treatment procedures. Based on quantitative behavioral analysis of video-recorded sessions, baseline compliance and/or comfort with each procedure was scored from 0 (unable to progress) to 10 (excellent compliance and relaxed comfort). Based on those results, 10 of the 26 horses were selected as five pairs that were matched for breed and the same two specific aversions. One of each pair was randomly assigned to alpha-casozepine supplementation (2,000 mg PO daily) and the other to control supplement for 5 days. On day 5 of supplementation, the standard battery of 12 procedures was repeated. For the five alpha-casozepine-supplemented subjects, compliance and/or comfort scores improved for seven of their 10 aversions compared with one of 10 for the five matched controls ($P < .01$, Fisher Exact). Average score increased 1.5 (standard error [SE] $\frac{1}{4}$ 0.87) points for alpha-casozepine aversions compared with an average decrease of 0.92 (SE $\frac{1}{4}$ 0.61) points for matched control aversions ($P < .05$, Wilcoxon signed ranks). These results indicate a modest benefit of alpha-casozepine supplementation to horses for improvement of compliance and apparent comfort with mildly aversive routine health care procedures.

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1. Introduction

The nutritional supplement alpha-casozepine, a benzodiazepine-like decapeptide derived from bovine milk

α -S₁ casein, has been found to have calmativ e anxiolytic-like properties in humans as well as several laboratory and domestic animal species, using a variety of stress models [1–5]. In rats, alpha-casozepine was found to have anxiolytic effects comparable with those of the benzodiazepine diazepam, both for the conditioned defensive burying and the elevated plus maze stress model scenarios [6,7]. In domestic pet cats presented for fear-related behavior problems, in a randomized, blind, clinical trial across multiple veterinary practices, oral alpha-casozepine

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treatment resulted in significantly greater client-reported improvement compared with placebo treatment [8]. In domestic pet dogs, in similar randomized blind clinical trials, alpha-casozepine treatment of anxiety-related disorders resulted in improvement in rating on a standard inventory of emotional disorders symptoms (Evaluation of Dog's Emotional Disorders, known as EDED) equal to that of the standard reference treatment selegeline [9]. In adult ponies undergoing transition from lifelong semiferal management to standard domestic housing and handling, alpha-casozepine-supplemented subjects progressed more rapidly with acclimation and training with fewer avoidance and stress behavior responses than matched-control counterparts [10].

To further explore the usefulness of alpha-casozepine supplementation in horses, the objective of the present study was to blindly evaluate the effects of alpha-casozepine on compliance and apparent comfort behavior of horses with established aversions to certain specific mildly aversive routine management and health care procedures.

2. Materials and Methods

2.1. General Procedure

A herd of 26 mares was evaluated for compliance and apparent comfort during a standard battery of procedures simulating mildly aversive routine management and health care examinations and treatments. From the 26 mares, 10 mares comprising five pairs matched for breed as well as for the two same specific aversions were selected for further study. Except for one of the two aversions for one of the five pairs, baseline rating scores were relatively similar (within three points on the 0–10 scale) for each of their two aversions. One of each pair was then randomly assigned to receive alpha-casozepine, and the other assigned to control supplement for five consecutive days. On day 5 of supplementation, procedure compliance and comfort were again assessed using the same standard battery of 12 procedures. Change in compliance and/or comfort rating score from the screening baseline to supplement day 5 reassessment for alpha-casozepine-supplemented and control-supplemented mares was compared.

2.2. Subjects

The 10 subjects (five matched pairs selected after initial evaluation of 26 mares), included two Standardbred and eight Thoroughbred mares, aged 3–13 years and weighing approximately 450–600 kg. These mares had been resident from 3 months to greater than 10 years at the University of Pennsylvania School of Veterinary Medicine in Chester County Pennsylvania for use primarily in teaching, research, and/or as embryo transfer recipients. They were maintained in pasture groups with supplemental hay as required to maintain good body condition. All subjects were generally familiar and compliant with basic ground handling and reproductive examinations involving a number of different caretakers and handlers of varying skill and experience levels typical of such a clinical training environment.

2.3. Specific Health Care Procedures

The standard assessment battery of routine health care procedures included, in order performed (1) entering as led into a small examination room (approximately 6 × 6.5 m), which was novel to the subjects, brightly illuminated with low sloped ceiling, novel rubber flooring, windows on three walls, and fluorescent lighting; (2) lifting each foot sequentially from left front counterclockwise to right front; (3) rectal thermometer insertion; (4) intramuscular needle stick (neck); (5) jugular needle stick; (6) oral examination; (7) oral medication; (8) eye examination; (9) eye medication; (10) intranasal application of saline; (11) lip twitch application; and (12) loading onto a stock trailer. Assessments were conducted from early to late afternoon. All procedures were video recorded.

The same equine veterinary technician consistently performed the procedures, whereas a second individual consistently assisted with handling the subject. Both were skilled and experienced with equine health care procedures and horse handling. The veterinary technician remained unaware of the purpose of the study or group assignments. The assistant, although aware of the purpose and design of the study, remained unaware of animal treatment assignments. This team had worked together handling horses for veterinary and health care procedures for more than 10 years. Neither had worked routinely with these particular mares. For each session, the technician led the mare from its pasture into the examination room using a cotton lead attached to the lower ring of the halter, whereas the assistant directed the video camera to maintain video view of the mare while entering the room. Once the mare was positioned in the center of the examination room, the lead was transferred to the assistant while the technician proceeded through the battery of examinations and treatments. At the completion of the examination and treatment procedures, the horse was taken by the examination technician and led from the examination room to a stock trailer positioned approximately 10 m outside the examination room entry and/or exit door, whereas the assistant repositioned the video camera to maintain view for continued recording of the trailer loading process. The assistant then stood to the side of the trailer to manage the stock trailer door. The handling manner for loading was a calm and confident positive enticement and reinforcement-based non-confrontational style routinely used by this technician-assistant team. After each session before the assessment of another subject, the examination room and trailer were cleaned and all equipment and supplies arranged in a standard fashion.

During the week of this study, the subjects remained pastured with their herd with no handling other than hand-feeding of supplements for this study and the baseline and reassessment examinations.

2.4. Supplements

Alpha-casozepine and control supplement commenced 2 days after the baseline behavior assessment. Alpha-casozepine supplementation consisted of 2,000 mg alpha-casozepine (Zylkene; Merck Animal Health, Summit, NJ)

once daily between 8 AM and 9 AM. The dose was selected based on recommended daily doses for the equine product of 1,000–2,000 mg, for horses weighing up to 500 kg body weight, and 2,000–4,000 mg, for horses weighing greater than 500 kg. Control supplementation consisted of an equal volume of oat powder ground to match the visual appearance of the alpha-casozepine powder. Supplements were sprinkled over 50 mL of Equine Senior pellets and fed from a hand-held 2-L rubber feed pan. Subjects typically licked the pans clean of powder particles. If necessary, additional feed pellets were scattered sparsely over any remaining powder until all powder was ingested. All animal interactions and data acquisition were done blindly to alpha-casozepine supplement or control group assignments.

2.5. Measures of Compliance and Comfort

2.5.1. Quantitative Scoring

Video recordings were viewed to derive for each of the 12 procedures: (1) the time (in seconds) required to complete (or abandon) the procedure; (2) the number and severity (mild, moderate, and severe) of each of any avoidance responses (head high or other evasive movement, kick, strike, side step, pull away from or push into handler, rear, and bolt) during the procedure; and (3) as an indication of comfort versus anxiety, fear, and/or stress response, the frequency of defecation with notation of fecal consistency (normally formed, loose, or watery) during the examination and/or treatment procedures phase and again during the trailer loading phase as well as the number of tail and ear movements, pawing, or snorting suggesting anxiety or agitation during each procedure. The behavior analysis technician remained unaware of the purpose of the study or group assignments. Based on these data, for each mare, a compliance and/or comfort score on a rating scale from 0 to 10 was assigned for each of the 12 procedures, using the scoring guidelines summarized in Table 1.

2.5.2. Handler Impressions

In addition to these video-derived quantitative measures, immediately after each mare assessment, the handler and technician each independently recorded on a standard form their subjective impressions of the subject's compliance and comfort with each of the 12 procedures.

Following the reassessment, each specifically noted their subjective judgment of any change from baseline.

2.6. Data Analysis

For each subject, for each aversion, change in compliance and/or comfort score from the baseline to the reassessment session was calculated. The nonparametric procedures, Fisher Exact test, and Wilcoxon signed ranks test were used to compare change in scores of alpha-casozepine-supplemented group aversions to those of their matched counterparts. Data analyses were completed without knowledge of alpha-casozepine and control group identities.

3. Results

3.1. Quantitative Scoring

Table 2 lists the six specific types of procedure aversions represented among the five matched pairs, along with the compliance and/or comfort scores for baseline assessment and supplement day 5 reassessment. Of the 10 paired aversions represented among the five alpha-casozepine subjects, from baseline to day 5 reassessment, seven of the 10 compliance and/or comfort scores improved, two remained the same, and one worsened. For the 10 aversions represented among the five control subjects, one improved, five remained the same, and four worsened. The difference in the proportion that improved is significant ($P < .01$; Fisher Exact). For the 10 aversions among the five alpha-casozepine subjects, the average compliance and/or comfort score increased 1.5 (standard error [SE] $\frac{3}{4}$ 0.87) points, whereas for control subjects, the average score decreased 0.92 (SE $\frac{3}{4}$ 0.61) points ($P < .05$; Wilcoxon signed ranks). The difference is significant ($P < .05$; Wilcoxon signed ranks test).

3.2. Handler Impressions

The technician's and assistant's subjective notations regarding compliance and comfort in each session and comments on change from baseline to supplement day 5 reassessment were generally in agreement with each other and with the quantitative scoring results. For four of the

Table 1
Operational definitions of compliance and/or comfort rating scores

Score	Progress With the Procedure	Total Frequency of Avoidance and Anxiety, Fear, and/or Stress Responses
0	None, with >60 s delay in spite of adjusted restraint and positive distractor(s) (scratching, food)	>25, some dangerous
1		<25, some dangerous
2	Minimal, with >60 s delay in spite of adjusted restraint and positive distractor(s) (scratching, food)	>20
3		>20
4	Completed with >20 s delay, prompting either adjustment of restraint or use of positive distractor(s)	>20
5	(scratching, food)	<20
6		>15
7	Completed with 10–20 s delay due to horse's behavior	11–20
8		<10
9	Completed without delay due to horse's behavior	0–5
10		None

Table 2

Baseline and supplement day 5 reassessment compliance and/or comfort rating scores for each of 10 pairs of aversions represented in five pairs of mares matched for two aversions per mare, same two aversions within pairs

Specific Procedure Aversion Type	Compliance and/or Comfort Scores					
	Alpha-Casozepine			Matched Control		
	Baseline (Mare)	Supplement Day 5	Change	Baseline (Mare)	Supplement Day 5	Change
Enter examination room	7 (a)	8	⊃ 1	6 (b)	0	□ 6
	8 (c)	9.5	⊃ 1.5	8 (d)	8.75	⊃ 0.75
Jugular stick	5 (a)	5	0	7 (b)	6	□ 1
	2 (e)	8	⊃ 6	7 (f)	7	0
Eye medication	7 (g)	8	⊃ 1	6 (h)	6	0
	6 (e)	10	⊃ 4	6 (f)	5	□ 1
Lip twitch application	7 (i)	9	⊃ 2	7 (j)	7	0
	5 (c)	5	0	2 (d)	0	□ 2
Trailer loading	6 (g)	2	□ 4	8.5 (h)	8.5	0
	5 (i)	9	⊃ 4	5 (j)	5	0
Mean (SE)	5.8	7.3	⊃ 1.5 (0.87)	5.8	5.3	□ 0.9 (0.61)

SE, standard error.

seven specific aversions that improved by one to two points, notations indicated uncertainty of the magnitude of change, for example “Most likely improved a bit.”

4. Discussion

These results indicate a modest benefit of alpha-casozepine supplementation to horses for compliance and apparent comfort with mildly aversive routine health care procedures. This is consistent with previous research in various models of fear or stress in other species and in ponies.

Within the horse industry, difficulties with these common management and health care handling procedures represent considerable concern not only for reduced efficiency in handling and caring for horses but also for human and animal safety and welfare. Among the herd of 26 mares initially assessed to identify matched pairs of aversions for this study, only five scored 9 or 10 on all 12 procedures. The remaining 21 exhibited moderate-to-severe aversion with one to seven of the 12 procedures. Although data-based estimates of the prevalence of specific procedure aversions in domestic horses have not been published, it is our clinical impression that these mares are likely typical of the domestic horse population at large. Although there are horses that have difficulties with handling in general, it is not uncommon for horses, such as these, to be otherwise well trained and compliant with most of the common management handling and health care procedures, but have difficulties with one or more specific procedures. Our clinical impression is specific aversions to relatively mildly aversive health care and management procedures typically represent learned avoidance responses as a result of specific negative experience associated with the procedure, often inadvertently reinforced natural avoidance behavior.

When applying an aid to behavior modification, it is helpful if improvement in compliance and comfort behavior is sufficient to be apparent to handlers or at least meets the handlers' expectations for rate of improvement. In this study, the improvement with supplementation, although significantly different from that of control subjects based on quantitative evaluation, was relatively modest in some cases. The technician and assistant

performing the procedures were occasionally uncertain of the improvement, although these handlers were skilled at behavior modification techniques for acclimation and/or rehabilitation of horses to these procedures within a clinical and research context that requires recognizing and recording incremental changes in behavior. This is in contrast to our experience in the previous clinical trial involving administration of alpha-casozepine to ponies during acclimation to domestic conditions and handling where alpha-casozepine-supplemented subjects rather consistently and conspicuously outscored all matched-control subjects [10]. At least two differences in the study design may have contributed to this apparent difference in consistently recognizable benefit. First, the dose rate of alpha-casozepine was higher in the pony study, 4.9–6.2 mg/kg daily compared with 3.3–4.4 mg/kg in the present study. In addition, the duration of daily supplementation was longer and the interval from daily feedings of supplement to handling and assessment was shorter in the pony trial. Evaluations of effects of alpha-casozepine supplementation in humans, for example, have been done after 30 days [1–4]. Further, the behavior models of the two trials differed in ways that may contribute to differences in response to various interventions and aids to behavior modification. In the pony study, relatively naive subjects with a history of limited human handling, which had all been based on positive reinforcement and avoiding negative experience with humans, were acclimated to human handling and routine health care procedures. In the present study, the behavior model was established aversions in domestic horse mares. The model of acclimation of naive subjects differs from that of rehabilitating established specific procedure aversions that have been learned as a result of negative experience associated with those procedures or as a result of inadvertent reinforcement of natural avoidance behaviors during life-long human handling and training for various uses. Certainly, further study of various dosing rates and schedules is necessary to establish maximum effectiveness for the various models of stress inherent to equine health care, management, or training to which it might be applied.

Although alpha-casozepine effects have been found in several mammalian species, both by intraperitoneal and

oral administration, the mechanism of absorption and action resulting in benzodiazepine-like effects without the typical benzodiazepine side effects are not fully understood. For the oral route of administration, current evidence indicates that ordinary digestive enzymes *in vitro* produce a bioactive fragment of the peptide with demonstrated anxiolytic effects in three standard behavioral models [11].

Acknowledgments

This is a Dorothy Russell Havemeyer Foundation Project. Alpha-casozepine (Zylkene) and partial funding were provided by Merck Animal Health. The study was designed and conducted and the manuscript written by S.M.M. with the technical assistance of animal health technician J.M. and in consultation with W.V. W.V. is an employee of Merck Animal Health.

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