ORIGINAL RESEARCH

REFEREED

Dietary Capsaicin Does Not Alter Synovial Concentrations of Prostaglandin E_2 or the Acute Phase Response in Aged Horses after Antigenic Challenge

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ABSTRACT

Dietary capsaicin enhances disease resistance and immunity in various species. Because relatively little is known about the potential benefits of capsaicin when used on horses, this study was conducted to determine the effect of dietary capsaicin on measures of health in horses. Twelve horses were fed over 28 days a basal diet with three levels of dietary capsaicin: 0 mg (C), 50 mg (CAP50), or 100 mg (CAP100) per horse per day. Before feeding on day 0, horses were weighed, a blood sample taken, and a sample of synovial fluid from the left distal carpal joint was taken. Subsequent body weights and blood samples were obtained on days 7, 14, 21, and 28. On day 21, tetanus toxoid (TT) and an immunomodulator (EqStim) were given to each horse. On days 21 to 28, daily rectal temperature (RT) and blood samples were taken. On day 28, synovial fluid was obtained immediately after blood sampling and RT measurement. Synovial concentrations of prostaglandin E_2 did not differ among dietary treatments or between days 0 and 28. No effect of dietary capsaicin on serum immunoglobulin G subclass T or α_1-acid glycoprotein concentrations was observed. Serum haptoglobin was elevated ($P < .0003$) and RT increased ($P < .05$) after challenge with EqStim and TT; however, haptoglobin concentrations and RT did not differ due to diet. We conclude that the doses of dietary capsaicin fed to horses in this study had no beneficial effect on measures of joint health or the immune response in horses.

Keywords: Capsaicin; Horse; Health; Prostaglandin; Acute Phase

INTRODUCTION

Capsaicin is the chemical responsible for the "heat" in chile peppers. Recent research has demonstrated that chile byproducts can be a useful source of nutrients for livestock.[1] Furthermore, these byproducts represent a source of capsaicin with little value to the food industry.

Several reports document the antimicrobial properties of capsaicin,[2] the ability of dietary capsaicin to enhance disease resistance in broiler chickens,[3] and the inhibitory effects of capsaicin on nitric oxide and prostaglandin E_2 (PGE_2) synthesis.[4] Others reported that dietary concentrations of 20 ppm capsaicin enhanced T-lymphocyte mitogen-induced proliferation, B-lymphocyte numbers, and the serum antibodies immunoglobulin G (IgG) and M (IgM) in mice.[5] Collectively, these data show the potential for capsaicin to modulate various arms of the immune system as well as the response to an antigenic challenge. However, published research studying the effect of capsaicin administration to horses is limited to a report in which topical application of capsaicin ointment to the palmar digital nerves provided measurable pain relief to horses.[6] Therefore, the objective of this study was to determine the effect of dietary capsaicin supplementation on health and immune function in the horse.

MATERIALS AND METHODS

In the absence of published reports in which capsaicin was fed to horses, three horses not included in the main study were observed in a pretrial period. Horses were fed 2 kg wet beet pulp mixed with 15 or 30 g dried jalapeno powder to determine which dose would prevent consumption of the feed during a 6-hour period. All three horses did not consume the mixture containing 30 g powder. However, all three horses ate the mixture containing 15 g powder, and two of the horses consumed the entire ration over a 6-hour period. Therefore, 15 g dried jalapeno powder as the maximum inclusion level, per feeding, was selected for this study.

Twelve horses of light horse breeding (3 mares and 9 geldings) were ranked by age (17.6 ± 0.2 years), and one of three treatment diets was randomly assigned to horses

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with one mare in each treatment. The treatment diets were: control (C), 1 kg of beet pulp pellets and 2 kg mid-bloom alfalfa hay (7:00 AM feeding), 2 kg mid-bloom alfalfa hay (12:30 PM feeding), and 1 kg beet pulp pellets and 2 kg mid-bloom alfalfa hay (5:30 PM feeding) with free access to trace mineral salt and water; control diet plus 50 mg capsaicin/head/d (CAP50); and control diet plus 100 mg capsaicin/head/day (CAP100). The CAP50 treatment supplied 6.75 ppm and the CAP100 treatment supplied 12.5 ppm capsaicin in the total diet, respectively. To ensure diet consumption throughout the trial, dry matter intake was limited to approximately 1.4% of body weight (BW). Beet pulp pellets were hydrated with 1 kg water before feeding, and dried jalapeno powder (3,385 ppm capsaicin) was mixed into the beet pulp immediately before feeding at 7:00 AM and 5:30 PM. Horses were individually fed in 3.7 × 3.7 m box stalls for 28 days and exercised daily at a trot on a mechanical hot walker for 20 minutes.

Before feeding on day 0, horses were weighed, a blood sample was taken via jugular venipuncture, and a sample of synovial fluid from the left distal carpal joint was taken for analysis of PGE_2. Subsequent body weights and blood samples were obtained on days 7, 14, 21, and 28 before feeding. On day 21, 1 ml tetanus toxoid (TT; Super-Tet, Intervet, Inc., Millsboro, DE) and 5 ml immunomodulator (EqStim, Neogen Corporation, Lexington, KY) were given to each horse. These biologicals are capable of stimulating B and T lymphocytes, respectively, to initiate an immune response.[7,8] On days 21 to 28, daily (7:00 AM) rectal temperature (RT) and blood samples were taken prior to feeding. Serum was assayed for haptoglobin (HAPT), α_1-acid glycoprotein (AGP), and immunoglobulin G subclass T (IgG(T)). On day 28, synovial fluid was taken immediately after blood sampling and RT measurement. All samples were stored at -20°C until assayed.

Analysis of Serum Proteins

Frozen serum samples were shipped on dry ice to the Kansas State University Comparative Hematology laboratory for analysis of HAPT according to the procedure of Smith et al.[9] Serum concentrations of IgG(T) and AGP were determined using commercially available radial immunodiffusion kits (Kit #448-60, VMRD, Inc., Pullman, WA, and Kit # P0501-1, Cardiotech Services, Louisville, KY).

Analysis of PGE_2 in Synovial Fluid

Frozen samples were shipped on dry ice to the Kansas State University Department of Animal Sciences and Industry Physiology Lab for analysis using a commercially available enzyme-linked immunosorbent assay for PGE_2 (Kit # 514010, Cayman Chemical Company, Ann Arbor, Michigan). The sensitivity of the assay was 7.81 pg/ml, and the intra-assay CV was 1.43%.

Statistical Analysis

Data were analyzed as a completely randomized design with repeated measures using the autoregressive covariance structure of the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). The model included effects of capsaicin level, day, and the interaction. Comparisons between treatments or times were made when a significant *F*-test ($P < 0.05$) for the main effect or interaction was found. Least-square means are reported.

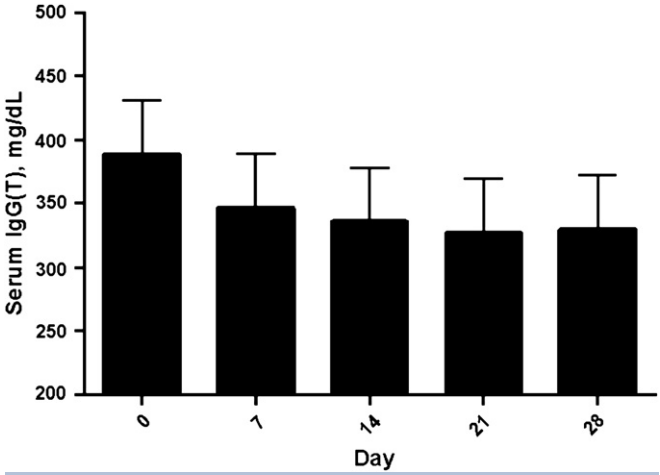

Figure 1. Serum immunoglobulin G (T) in horses (n = 12) before and after challenge on day 21 with tetanus toxoid and EqStim. Means do not differ ($P > .15$).

RESULTS

Horses required approximately 2 days on trial to become accustomed to consuming the daily ration containing capsaicin. By monitoring feed intake, we observed that some horses had no aversion to the beet pulp-capsaicin mixture and others refused to eat the entire daily ration. This was overcome by mixing some of the daily alfalfa ration into the beet pulp–capsaicin mixture to insure that each horse consumed its daily dose of capsaicin. No symptoms of colic or changes in fecal consistency were observed. The predominant behavioral change noted was excess salivation in some horses and increased wood chewing of the stalls. This may have been a response to the dietary treatment; however, wood chewing was noted even in control horses, which may have been indicative of boredom or limited feed intake.

Body weight of horses increased ($P < .0001$) over the course of the trial such that BW on days 14 (539.86 kg), 21 (530.05 kg), and 28 (531.61 kg) were greater ($P < .05$) than BW on day 7 (524.30 kg) and day 0 (518.35 kg; pooled SEM ± 17.19 kg). Body weight on day 7 was also different ($P < .05$) from day 0, but BW did not differ between capsaicin treatments. Synovial concentrations of PGE_2 did not differ among C (58.02 ± 35.80 pg/ml), CAP50 (79.08 ± 35.27 pg/mL), and CAP100 (68.94 ± 35.80 pg/mL), and synovial PGE_2 did not differ between days 0 and 28 (71.13 and 66.23, respectively ± 21.69 pg/mL). No effect of dietary capsaicin or day on serum IgG(T) concentrations was detected (Fig. 1). Serum HAPT was