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Original Research

A Pilot Study on the Effects of Curcumin on Parasites, Inflammation, and Opportunistic Bacteria in Riding Horses

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ABSTRACT

Twelve riding horses were used to examine the effects of curcumin on intestinal parasites, inflammation, and the fecal shedding of *Streptococcus bovis/equinus* complex (SBEC), *Clostridium difficile*, and *Clostridium perfringens*. Known for having anti-inflammatory, antimicrobial, and antiparasitic properties, it was hypothesized that curcumin would decrease parasite shedding, inflammation, and opportunistic bacteria found in the gastrointestinal tract of riding horses. Horses were randomly assigned to one of the following treatments (n = 6 per treatment): (1) no curcumin, control; or (2) 15 g of 95% pure curcumin (CUR). Curcumin was dosed per day for 30 days. Fecal samples were evaluated for shedding of ova and concentrations of selected bacteria. Blood samples taken pre- and postriding intervals and evaluated for erythrocyte sedimentation rate (ESR) for inflammation. All data were analyzed for repeated measures. Treatment had no effect ($P \geq .58$) on total fecal egg count, strongyles, or ascarids. Treatment had no effect on ESR ($P \leq .42$); however, ESR decreased ($P = .0006$) on day 14 in CUR horses. Treatment had no effect ($P \geq .34$) on concentrations of SBEC, *C. difficile*, or *C. perfringens*. Curcumin was not an effective compound against intestinal parasites or fecal microbial strains examined when administered for 30 days; but could potentially decrease inflammation. Curcumin has been observed to have many beneficial effects in other species; however, more research is needed to evaluate those benefits in horses.

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1. Introduction

Horse intestinal parasites pose an economic and health risk that are of concern to both breeders and horse owners [1]. The body of a horse is host to millions of microscopic organisms who use the horse's oxygen, nutrients, and body heat for survival. Parasites, such as *Strongylidae* (strongyles) can cause emaciation and anemia; whereas ascarids are known to cause a blockage in the intestines, which if not taken care of properly can lead to death [2,3]. Moreover, parasites, which can be found in the intestines of horses of all breeds, both sexes, and all age classes [4], can cause inflammation within the gastrointestinal tract (GIT).

In addition to parasite-induced inflammation, inflammation can also occur because of the athletic lifestyle required of domesticated horses. Repetitive stress applied to the joints from speed work, jumping, and extreme hindquarter thrust, results in inflammatory changes to the bone structure, joint anatomy, and synovial fluid as well as predisposed factors. Although some horses are diagnosed with lameness in their younger years, many develop the progressive problem over time, whether it is mild or severe [5]. Therefore, inflammation in a horse can be due to several factors, including illness, injury, GIT parasites, and even an altered hindgut microbiome.

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Ethical approval statement: The study was reviewed and approved by the Office of Sponsored Projects Administration (OSPA) at Southern Illinois University.
Conflict of interest statement: The authors declare no conflicts of interest.

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The gut microbiota is one of the densest, most dynamic, and complex microorganism populations located in the body [6]. Gut microbiota act against pathogens, aid in digestion and absorption, and stimulate the immune system [7,8]. If the microbiome is altered, this could result in gastrointestinal diseases, such as enterocolitis, diarrhea, ulcers, anorexia, colic, and even death [9,10]. *Streptococcus bovis/equinus* complex (SBEC), *Clostridium difficile*, and *Clostridium perfringens* are bacteria found in the hindgut microbiome that are considered opportunistic because of GIT issues when the immune system is compromised. *Streptococcus bovis/equinus* complex is a group of human- and animal-derived streptococci that are commensal, opportunistic pathogens, or food fermentation associates [11]. *C. difficile* is commonly associated with the onset of colic in horses, but has also been isolated from foals with diarrhea. *C. perfringens* causes enterocolitis in neonatal foals; in addition, this species produces endotoxins that can cause diarrhea and severe damage to the mucosa [9]. When compared with other mammals, little research has been conducted on the microbiota in the GIT of horses [9].

Curcumin is the active ingredient in turmeric (*Curcuma longa*) that is not only known for having anti-inflammatory properties, but also possessing antimicrobial, wound healing, and antiparasitic properties [12,13]. In addition to curcumin having many biological activities, it is relatively safe and well-tolerated [14]. Testing curcumin has shown effective antiparasitic properties, it was an effective compound against *Raillietina cesticillus* in birds [12], strongyles in cattle [15], and fecal egg shedding in goats [16]. The indication of the safety and efficacy of curcumin provided a solid basis for evaluating its antiparasitic and antimicrobial properties in riding horses. We hypothesized that curcumin would decrease parasite shedding, inflammation, and opportunistic bacteria found in the GIT of riding horses. The main objectives were to evaluate fecal shedding of intestinal parasite ova and selected opportunistic bacteria as well as erythrocyte sedimentation rate (ESR) when dosing curcumin at 15 g per day for 30 days to riding horses.

2. Materials and Methods

Twelve horses, 10 Southern Illinois University of Carbondale owned riding horses and two privately owned riding horses were used for this study. All horses were between the ages of 5 and 20 years, and did not have any concurrent illnesses and/or ailments; they also did not receive any medications or dewormer for 30 days before the commencement of this research trial. The predominate breed used was Quarter Horse (nine), one mustang, one warmblood, and one draft horse. Care and handling of animals used in this study was approved by Southern Illinois University Animal Care and Use Committee (Protocol 15-041).

Horses were randomly assigned to one of the following treatments: (1) no curcumin (CON) or (2) 15 g of 95% pure curcumin (CUR). The average age of the CON horses was 12.5 $\pm$ 7 years, whereas the average age of the CUR horses was 13.5 $\pm$ 7 years. There were three gelding and three mares on CON and six mares on CUR treatments. The CON horses received 3–4 alfalfa cubes, moistened with water, and mashed. The CUR horses received the same alfalfa cube mash as CON horses, but with 15 g of curcumin (Noble Elephant Supplement, San Dimas, CA) mixed into the mash. The dosage of curcumin was based on the recommended dose of one tablespoon, which equates to 15 g per horse [17]. Horses were gathered and samples were collected at 11 AM daily. Once samples were collected, horses were fed either CON or CUR treatment. All horses were housed on pasture and grazed ad libitum when not ridden. All horses were ridden for an average of 3 hours daily for 4 days a week.

2.1. Analysis of Intestinal Parasites

A fresh fecal sample was collected from each horse at 11 AM, before initiation of the study (day 0), and then daily for 30 days. Fecal samples were collected, placed in a Whirl-Pak sample bag (Nasco, Fort Atkinson, WI), then placed in the refrigerator. Once all samples were collected (approximately 1 hour later), 3 g of feces was weighed and the remainder of sample was immediately frozen at -20°C for later analysis of opportunistic bacteria. Fecal parasite load was determined using the modified Wisconsin sugar flotation method and the centrifugation technique with a specific gravity between 1.20 and 1.33; strongyles, ascarids, and total eggs were counted and recorded [18,19].

2.2. Erythrocyte Sedimentation Rate

Blood samples were collected via jugular venipuncture using a 5 mL syringe with a 20-gauge, 1.5-inch needle and transferred to a 5 mL vacutainer K2 ethylenediaminetetraacetic acid tube (Fischer Scientific, Franklin Lakes, NJ) for ESR assessment (Globe Scientific, Paramus, NJ). The Westergren ESR test was performed on days 0, 3, 7, 10, 14, 17, 21, 24, and 28 to examine inflammation pre- and postriding. Samples collect on days 0, 7, 14, 21, and 28 were collected before riding. Samples collected on days 3, 10, 17, and 24 were collected after 4 days of riding in which horses were ridden for an average of 3 hours per day.

2.3. Fecal Analysis of Opportunistic Bacteria

2.3.1. Growth of Bacteria

Pure cultures of selected opportunistic bacteria were grown and used as standards for quantitative polymerase chain reaction (qPCR). Trypticase soy broth (30 g/L) and yeast extract (3 g/L) medium was made for SBEC, according to Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Germany) media recipes. *Clostridium* medium (17 g/L digest casein, 3 g/L digest soy, 5 g/L NaCl, 2.5 g/L K_2Pho4, and dextrose) was made for both *C. difficile* and *C. perfringens*, according to Difco (Becton, Dickson and Company, Sparks, MD). Ten milliliter of broth was pipetted into glass Hungate tubes and deoxygenated with nitrogen. Rubber stoppers and metal caps were crimped on the tubes and then were autoclaved at 121°C, 15 psi, for 15 minutes. Hungate tubes were inoculated with pelleted strains of bacteria, *C. difficile*, *C. perfringens*, and SBEC. Dense bacterial samples were transferred to a new Hungate tube every 3 days for 10 days to ensure pure cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH