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

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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 utilized 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 GIT of riding horses. Horses were randomly assigned to one of the following treatments (n = 6/treatment): 1) no curcumin, control (CON); or 2) 15 g of 95% pure curcumin, (CUR). Curcumin was dosed per day for 30 d. Fecal samples were evaluated for shedding of ova and concentrations of selected bacteria. Blood samples taken pre and post riding intervals and evaluated for erythrocyte sedimentation rate (ESR) for inflammation. All data were analyzed for repeated measures. Treatment had no effect ($P \geq 0.58$) on total fecal egg count, strongyles, or ascarids. Treatment had no effect on ESR ($P \leq 0.42$); however, ESR decreased ($P = 0.0006$) on d 14 in CUR horses. Treatment had no effect ($P \geq 0.34$) 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.

Keywords: Curcumin; Equine; Inflammation; Opportunistic bacteria; Parasites

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 utilize the horse's oxygen, nutrients, and body heat for survival. Parasites, such as *Strongylidae* (strongyles) can cause emaciation and anemia; while 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 due to 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.

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 due to GIT issues when the immune system is compromised. SBEC 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 to 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 *R. 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 when dosing curcumin at 15 g per day for 30 days to riding horses.

2. Materials and Methods

Twelve horses, ten Southern Illinois University of Carbondale (SIUC) owned riding horses and two privately owned riding horses were used for this study. All horses were between the ages of five and twenty years old, and did not have any concurrent illnesses and/or ailments; they also did not receive any medications or dewormer for 30 days prior to the commencement of this research trial. The predominate breed utilized 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 years old ± 7 years, while the average age of the CUR horses was 13.5 ± 7 years. There were 3 gelding and 3 mares on CON and 6 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 off the recommended dose of one tablespoon, which equates to 15 g per horse [17]. Horses were gathered and samples were collected at 1100 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 three hours daily for four days a week.

2.1 Analysis of Intestinal Parasites

A fresh fecal sample was collected from each horse at 1100, prior to initiation of the study (d 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 one 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 to 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 erythrocyte sedimentation rate (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 post-riding. Samples collect on days 0, 7, 14, 21, and 28 were collected prior to riding. Samples collected on days 3, 10, 17, and 24 were collected after four days of riding in which horses were ridden for an average of three hours per day.

2.3 Fecal Analysis of Opportunistic Bacteria

Growth of Bacteria

Pure cultures of selected opportunistic bacteria were grown and used as standards for 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 (DSMZ) (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 mL 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 min. 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 three days for ten days to ensure pure cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) (Germany)). These pure cultures contained the forward and reverse primers in Table 1.

DNA Extraction

DNA was extracted from the daily fecal samples collected using PowerFecal Mo Bio DNA Extraction Kits (Mo Bio Laboratories, Carlsbad, CA). The pure cultures that were used as standards for qPCR (Bio-Rad MyiQ Optical System Software 2.0) were extracted using PowerFecal Mo Bio DNA Extraction Kits (Mo Bio Laboratories, Carlsbad, CA) and then purified using UltraClean 15 DNA Purification Kits (Mo Bio Laboratories, Carlsbad, CA). All DNA extractions were assessed for concentration using a Nano Drop ND-1000 Spectrophotometer (Wilmington, DW).

Real Time qPCR

Real-time qPCR reactions were performed in triplicate using a MyIQ thermocycler (BioRad, Hercules, CA) in a total volume of 25 µL. The reaction mixture was composed of Maxima SYBR Green/ROX qPCR (Thermo Scientific, Waltham, MA), the corresponding forward and reverse primers (Eurofins MWG Operon, Louisville, KY) (Table 1), extracted DNA, and sterile water. The thermal cycling protocols for *C. difficile* [20], *C. perfringens* [21], and SBEC [22] were utilized at an average starting concentration of DNA at 52 ng/µL, 20 ng/µL, and 17 ng/µL, respectively.

2.4 Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS 9.4 Inst., Inc., Cary, NC) for repeated measures. The model included treatment, day, and treatment × day interactions. An autoregressive covariance structure (AR1 of the MIXED procedure of SAS) was determined to be most appropriate based on Akaike's Information Criterion. There were no treatment × day interactions; therefore, only treatment means are reported. Comparisons of main effects were

133 determined using least square means and Fisher's protected LSD ($P \leq 0.05$) and a trend set at (P
134 ≤ 0.10).

135 **3. Results and Discussion**

136 ***3.1 Analysis of Intestinal Parasites***

137 Dosing curcumin at the recommended rate of 15 g per horse had no effect ($P \geq 0.58$) on
138 the amount of shedding of total fecal egg count, strongyles, or ascarids; however, there was a day
139 effect. Total egg counts for both CON and CUR horses decreased ($P \leq 0.05$) from d 1 to 10 then
140 increased from d 11 to 19 followed by a decrease from d 20 to 29 with a substantial increase on d
141 30. This pattern of decreasing and increasing ova was due to both strongyles ($P = 0.05$) and
142 ascarids ($P = 0.0007$) decreasing and increasing for both CON and CUR horses. The increase
143 and decrease of shed ova, approximately every ten days, would suggest the pattern of the
144 strongyles lifecycle was observed [23]; these intestinal parasites can develop from an egg to
145 larval stage 3 in a period of around one week in the optimal temperatures (8 – 38°C). The study
146 was conducted at the onset of spring when it is expected for fecal egg counts to be higher than in
147 the winter months [23, 24]. In contrast, when dosing cattle turmeric at 100 mg/ml a decrease in
148 strongyles was observed [15]. The same dose was also determined to be 100% effective against
149 adult gastrointestinal tract nematodes (roundworms) [15]. Similarly, a decrease in fecal egg
150 shedding was observed when goats were dosed 0.2% or 0.6% curcumin of the daily ration for 60
151 days when compared to d 0 [16]. In the current study, it is possible that 30 days was not a long
152 enough dosing period or that a larger dose is needed to observe the antiparasitic effects of
153 curcumin. It should also be noted that treatments in the current study were randomly assigned
154 prior to d 0 fecal egg counts and unfortunately most of the high shedding horses were randomly
155 assigned to CUR, which could have influenced the results. The results would suggest that dosing

curcumin for 30 days is not effective for intestinal parasite control in riding horses during the spring months of the Midwest region and that further research is needed to examine effects of curcumin with a longer dosing period or at an increased dose. In the current study, curcumin was utilized based on previous parasite work, mentioned above, and also do to its lower cost to supplement. However, the bioavailability of curcumin is noted to be minimal due to being hydrophobic, low intrinsic activity, poor absorption, and high rate of metabolism and elimination from the body [25]. It is possible that the results would have been different had liposomal curcumin been utilized. In rats, oral administration of liposome-encapsulated curcumin showed increased bioavailability of curcumin [26, 27].

3.2 Erythrocyte Sedimentation Rate Analysis

There were no treatment x time interactions and treatment had no effect on ESR ($P \leq 0.42$); however, a day effect ($P \leq 0.001$) was observed (Table 2). ESR within the CUR horses, significantly decreased ($P = 0.0006$) on d 14 and again on d 21 ($P = 0.02$) compared to d 0 (Figure 1). However, there was no difference ($P = 0.64$) between d 0 and d 28. There was no day effect ($P = 0.29$) for ESR in CON horses. This would suggest that it would take at least 14 days before curcumin could potentially decrease inflammation in riding horses. These findings are similar to Farinacci et al. (2009) [28], who observed curcumin down regulating COX-2, TNF- α, and IL-6 when mares and foals were administered 4mg/kg of CURCUVET®; and on d 15, COX-2 was significantly down-regulated. However, in the current study inflammation on day 28 was not significantly different compared to day 0 which would further suggest that the bioavailability of curcumin is minimal, as previously discussed. Moreover, factors such as an increase in ambient temperature, experience of the rider and saddle fit could have contributed to a similar ESR on day 28 compared to day 0.

3.3 Fecal Analysis of Opportunistic Bacteria

There was no treatment ($P = 0.34$) or day effect ($P = 0.53$) on concentration of *C. perfringens* (Table 2). Similarly, there was no treatment effect for *C. difficile*, ($P = 0.51$) or SBEC ($P = 0.69$). However, *C. difficile*, for both CON and CUR horses, had a significant day effect ($P = 0.0001$) with all horses having higher concentrations on d 0 and d 1 compared to all other days. Furthermore, concentrations of SBEC were affected by day ($P = 0.05$) with CON and CUR horses having similar concentrations at d 0 and d 1. However, the CON horses had an increase in SBEC concentration on d 9, 25, 27, and 30 when compared to d 0, while the CUR horses had increased concentrations on d 9, 17, and 27 compared to day 0. The data from this study would indicate that curcumin can be dosed at 15 g per day for 30 days with no adverse side effects on opportunistic bacteria concentrations, however, more research is needed to evaluate the antimicrobial properties of curcumin when dosed to horses over a longer duration and at varying dosages.

5. Conclusions

The antiparasitic and antimicrobial properties of curcumin were not observed when 15 g of curcumin was orally dosed to twelve riding horses for 30 days. The inability for curcumin to decrease the parasite shedding load would suggest that curcumin will need to be dosed for longer periods of time or at higher dosages, if utilizing for intestinal parasite control. However, it is possible that curcumin can decrease inflammation after 14 days of administration. More research is needed to further evaluate the benefits of supplementing curcumin to horses, especially for intestinal parasitic control.

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Table 1. Forward and Reverse Primers used for real time PCR

Strains	Forward Primers (5'-3')	Reverse Primers (5'-3')
SBEC[1]	GCCTACATGAAGTCGGAATCG	TACAAGGCCCGGGAACGTA
C. difficile[2]	CAAGTTGAGCGATTTACTTCGGTAA	CTAATCAGACGCGGGTCCAT
C. perfringens[3]	AAATGTAACAGCAGGGGCA	TGAAATTGCAGCAACTCTAGC

[1]*Streptococcus bovis/equinus* complex [22]

[2]*Clostridium difficile* [20]

[3]*Clostridium perfringens* [21]

Table 2. Effects of 15 g of 500 mg/g of 95% curcumin dosed once daily to riding horses on fecal parasite load (number of eggs/3 grams of feces), erythrocyte sedimentation rate, and fecal opportunistic bacteria.

	Treatments[1]			***P*-Value**	
Parameters	**CON**	**CUR**	**SEM**	**TRT**	**DAY**
Total Egg	127	156	37.8	0.58	0.05
Strongyles	123	153	37.7	0.58	0.05
Ascarids	3.22	3.11	0.24	0.74	0.0007
ESR[2], mm/hr	89.6	94.5	4.21	0.42	0.001
SBEC[3], ng/μL	1.93	1.66	0.46	0.69	0.04
C. difficile, ng/μL	4108	8080	4136	0.51	0.0001
C. perfringens, ng/μL	0.01	0.0001	0.01	0.34	0.53

[1] Treatments: CON= control; CUR= curcumin dosed at 15g/day

[2] Erythrocyte Sedimentation Rate

[3] *Streptococcus bovis/equinus* complex

Figure 1. Effect of curcumin on Erythrocyte Sedimentation Rate over time (days).