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The Effects of Exercise on the Microbial Metabolites of the Equine Cecum

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Abstract:

Little is known about the effect of exercise on the microbial profile of the equine cecum. As hindgut fermenters, horses are particular sensitive to gastrointestinal disorders. Previous work (McKenzie et.al., 2010; Walshe and Duggan, 2011; Schoester et.al., 2012; and Jager et.al., 2013) has demonstrated an impact of exercise on the fecal microbial composition of equines and canines. However, no information has been reported on the effects of exercise related to ammonia and volatile fatty acid production within the cecum. The objective of this research was to test the hypothesis that increasing exercise would impact the production of ammonia and volatile fatty acids within the cecum. Four cecally-cannulated horses were used in a Latin square 4x4 to investigate the effect of increasing levels of exercise on cecal metabolites. Four exercise treatments (1 = no exercise; 2 = 5 minutes trot; 3 = 15 minutes trot; 4 = 20 minutes trot) were applied to test our hypothesis. Exercise was conducted by lunging with trained handlers. All horses were fed to maintain body condition score (BCS) = 5 ±1 and were weighed weekly with mean BW of 521 kg ± 24 kg. Each horse was given daily turnout for 8 ± 1 hrs and were stalled overnight in identical 3 x 4 meter stalls with *ad lib* access to water, salt, and 2.27 kg of mixed grass hay. Horses were fed pelleted complete grain (Strategy® Purina Mills, St. Louis, Missouri) twice daily at approximately 6:30 AM and 4:00 PM. Cecal samples were collected, each period, on day 1 prior to exercise and on day 7 following exercise. Data were analyzed using Proc Mix of SAS (v9.4 SAS Institute, Inc.) with significance established at ($P < 0.05$). Chemical analysis of cecal contents demonstrated no significant difference in ammonia or volatile fatty acid concentration across treatments. Further work should investigate the impacts of longer and more frequent exercise periods with greater intensity.

INTRODUCTION

Success in the equine industry is entirely dependent on performance. Thus, an understanding of how different exercise levels affects digestion can help owners to properly feed and maintain their animals. According to previous research (McKenzie et.al., 2010; Walshe and Duggan, 2011; Schoester et.al., 2012; and Jager et.al., 2013) the microbial population of the hind gut is impacted by exercise in correlation with amount and severity of the exercise that the animals underwent. Research has been previously performed on canines and equines through fecal sampling, however there is no data available on the impact of cecal microbial parameters as affected by exercise.

Streptococcus equi is one of the bacteria strains tested in this experiment. This bacterium is commonly associated with equine strangles. This disease is very serious and can travel quickly; therefore, it is important to know how to manage *Streptococcus equi*. These pathogenic microbes do not survive long in an aerobic environment. However, they can be carried asymptomatically within the equine digestive tract for long periods of time (Walshe and Duggan, 2011). One way that these microorganisms can be kept under control is by testing for them in a manner that is accurate and dependable. It has been shown in previous studies that polymerase

chain reaction (PCR) testing was used in the experiment as an efficient testing method that will detect dead or living microbes (Walshe and Duggan, 2011). Detection of these microbes is important to determining population changes during analysis.

Clostridium perfringens and *Clostridium difficile* are the other two bacteria being studied. Most of the information collected on these is represented collectively. These microbes are responsible for a broad range of intestinal upsets. *C. difficile* is found in multiple compartments of the digestive tract, however *C. perfringens* is usually only found in horses that are experiencing digestive upset (Schoster et.al., 2012). The presence of both opportunistic bacteria is associated with swelling in different sections of the colon (Schoester et.al., 2012). Using these two statements the supposition has been made that greater numbers of *C. perfringens* in the sample means that the horse has experienced a stressor (either environmental or physiological) such that it has resulted in intestinal upset. If there is a lower concentration of *C. perfringens* and *C. difficile* the horse is less likely to have digestive upset (Schoester et.al., 2012). One complication that may occur during sampling is that the colonization of the bacteria is not homogeneous throughout the intestine (Schoster et.al., 2012). Since the samples for this project were taken only from the cecum, the absence of the bacteria in the samples does not mean there is a complete absence of said bacteria in the intestinal tract.

Research with equines regarding the effects of exercise on the microbial content of the cecum is limited. The hypothesis for this experiment was developed from ideas generated from a previous report using sled dogs where tests were conducted for changes in intestinal bacterial concentrations. The conclusion of one study was that the stress associated with running the races resulted in the increase of *Strep equi* (Jager et.al., 2013). If horses have a similar reaction, then then an increased load of S*trep equi* would be expected when subjected to an exercise regimen. However, length and nature of exercise must be taken into consideration. These horses are not running a race that is miles long over many days and therefore results may differ.

In another study conducted utilizing sled dogs regarding *C. perfringens* and *C. difficile,* researchers determined that the presence of diarrhea, a symptom of intestinal upset, is not associated with the bacterial load (McKenzie, et.al. 2010). Since the conclusion of the this research was based off the fact that the dogs were having diarrhea, then it may be possible that changes in these microbes are not always caused by exercise, like the diarrhea noted. In fact sometimes in this study the concentration of *C.perfringens* and *C.difficile* were decreasing even though the amount of exercise was still high (McKenzie, et. al. 2010).

This project was designed to identify changes in microbial parameters associated with volatile fatty acid (VFA) and ammonia (NH_3) production. These fatty acids are the byproduct of microbial digestion and changes in microbial profile would be expected to result in changes in VFA production. Concentrations of VFAs in the cecum are correlated to bacteria concentrations in the cecum and cecal pH (NRC, 2007). Previous studies demonstrated that an increase in the short chain VFA concentrations in the hind gut are consistent with an increased level of bacterial fermentation (NRC, 2007). This correlation indicates increasing VFA concentrations are directly related to the concentrations of bacteria and the amount of material they are consuming.

Production of VFAs in the cecum accounts for 30% of the energy needs for a horse that is being fed at maintenance levels. However, if a horse is on a maintenance diet that is primarily hay, then 80% of energy production can come from VFAs in the cecum and colon (NRC, 2007). Data such as this is important to identify the horse's dependence on this unique energy source. The following calculations are the offered to determine maintenance levels of energy: maintenance = $BW^{0.75}$ to $BW^{0.67}$ (NRC, 2007). Once you have determined the energy

requirements for the horse at maintenance then you take 30% of that and that is the amount of VFA production that should be present in that horse's cecum during sampling.

Ammonia concentration is a function of protein catabolism and is also indicative of proteolytic bacteria (NRC, 2007). Ammonia is the byproduct from nitrogen, and it is absorbed in the hind gut of horse (NRC, 2007). The amount that is being absorbed in the cecum and colon is determined by the amount of protein breakdown that is taking place (NRC, 2007).

Currently, no research has been published that can identify changes in VFA and ammonia levels in the cecum as impacted by exercise. However, we do know that changes in these levels are positively correlated with changes in microbial population's level in the cecum (NRC, 2007).

METHODS

Animal Unit

Institutional Animal Care and Use Committee (IACUC) approval was obtained prior to initiation of this study. All research was conducted at Southern Illinois University (SIU) Equine Center, Carbondale, Illinois. Four, SIU-owned horses (3 mares and 1 gelding), age 8 ± 3 years with mean body weight of 521 ± 24 kg, up to date with vaccinations and in good dental health were used for this study. Each horse was turned out daily for 8 ± 1 hrs to a grass pasture for adequate social interaction. Horses were stalled overnight in identical 3 x 4 meter stalls with *ad lib* access to water, salt, and 2.27 kg of mixed grass hay. Horses were fed pelleted grain (Strategy® Purina Mills, St. Louis, Missouri) twice daily at approximately 6:30 AM and 4:00 PM to maintain a BCS 5. Weights were taken with a digital livestock scale and recorded once weekly for each treatment period.

Treatments

Treatment Name	Exercise Regimen
1	5 minutes walk, 0 minutes trot, 5 minutes walk
2	5 minutes walk, 5 minutes trot, 5 minutes walk
3	5 minutes walk, 15 minutes trot, 5 minutes walk
4	5 minutes walk, 20 minutes trot, 5 minutes walk

Treatment periods were seven days with a seven day withdrawal period following each. This withdrawal period was to allow the microbial populations to normalize before exposing the horse to the next treatment period. Horses were lunged by trained handlers each day in either a round pen or an arena. The same long line was used in order to make the size of the circle constant between all horses and treatments.

Samples were collected on day 1 of each treatment period (baseline) and day 7. Samples were collected directly through the cecal fistula and were aliquoted into three 15 ml sterile conical tubes and frozen for further analysis. Vitals (heart rate, rectal temperature, gut sounds, capillary refill) were also taken at the beginning and the end of each sampling period.

Pre-Exercise Sample Period	Exercise ONLY Days					Post-Exercise Sample Period
1	2	3	4	5	*6*	*7*
Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	*Friday*

Statistical Model

The horses were assigned to a treatment regime using a 4x4 Latin Square arrangement. This was to assign the treatments in a random order. Randomness assures that the changes in microbial profile were not due to the order that the treatments were administered, but the actual treatment itself.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Week 1	A	B	D	C
Week 2	B	A	C	D
Week 3	C	D	B	A
Week 4	D	C	A	B

Analysis

Cecal samples were divided into three 15 ml aliquots to prevent damage associated with repetitive freezing and thawing, and were stored at -8 □ F until further analysis. Samples were analyzed for VFA (Volatile Fatty Acids in Rumen Fluid, Clemson University, Clemson, SC) (GC-2010 Gas Chromatograph, Shimadzu, Japan) , ammonia (Ruminal Ammonia Assay, Chaney and Marbauch, 1962) (Spectronic Genesys 5 (Milton Roy Company, Ivyland, PA), and microbial profile. Cecal samples were analyzed by performing DNA extraction and purification (MO Bio PowerSoil Kit, MO Bio Laboratories, Carlsbad, CA), amplicon library prep with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), and next generation pyrosequencing (MiSeq, Illumina, San Diego, CA).

Statistical Analysis

VFA, ammonia levels, and vitals were analyzed using proc MIXED procedure of SAS (SAAS 9.4 Inst.Inc., Cary, NC) using the model for a Latin square design. The model included treatment and period with animal specified in the RANDOM statement of SAS. Significance for all parameters was set at $(P<0.05)$.

RESULTS

Volatile Fatty Acid Concentrations: (mean values) (mM)

Treatment	Acetate		Proprionic		Isobutyric		Butyric		Isovaleric		Valene		TOTAL	
	Pre-	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*
1	9.32	6.55	7.15	4.13	0.92	1.31	3.38	1.98	0.53	0.21	0.67	0.43	19.06	14.23
2	8.15	8.20	6.75	4.79	0.15	0.50	3.10	2.04	0.42	0.10	0.39	0.37	14.53	16.30
3	6.28	7.04	6.54	18.38	0.37	2.13	2.11	2.48	0.23	0.18	0.48	0.47	14.75	28.30
4	5.86	3.64	4.61	5.19	0.16	0.18	1.90	2.11	0.18	0.10	0.69	0.42	13.08	11.77

Volatile fatty acid concentrations were measured at the beginning and end of each treatment period. Significance for VFA concentrations was established at $P<0.05$.

Ammonia Concentrations: (mean values)

Treatment	Ammonia Concentration (mg/dL)	
	Pre-	*Post-*
1	0.19456744	0.16008174
2	0.1747701	0.18094346
3	0.23756812	0.16455211
4	0.17817609	0.18051771

Ammonia concentrations were measured at the beginning and end of each treatment period. Significance for ammonia concentrations was established at $P<0.05$.

Vitals: (mean values)

Treatment	Pulse (bpm)		Temperature (°F)		Respirations (bpm)		Gut Sounds (present/absent)		Capillary Refill (seconds)	
	Pre-	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*	*Pre-*	*Post-*
1	43	38	99.95	99.275	19	17	Present	Present	1.5	1.625
2	34	43	99.6	100.3	15	27.5	Present	Present	1.5	1.5
3	36	38	100.2	100.4	16	26	Present	Present	1.125	1.875
4	40	36	99.9	100.45	17	28	Present	Present	1	1.25

Individual horse vitals were collected at the beginning and end of each treatment period and consisted of temperature, capillary refill, gut sounds, respiration and pulse rate. No statistical difference was noted for vital signs recorded.

DISCUSSION

One must question whether or not the exercise regimen utilized in this study was sufficient to result in a microbial impact. This project should be repeated utilizing a treadmill or

other automated exercise equipment. Treadmills in this experiment would allow for consistency and faster speeds. On a long line the animals are all trotting. However, it is not possible to make sure that they all trot at a consistent speed. Electronic exercise equipment would also allow for horses to be exercised at a trot, canter and/or gallop. Higher speeds are more indicative of a horse in regular under saddle exercise session, because they commonly include walk, trot, and canter.

A treadmill also has the ability to be run at different inclines. Including this in a future study would allow the subject to mimic that of a horse that is taking part in endurance training. As seen in previous research the strain of long events such as sled dog racing has the ability to change microbial populations in the digestive tract of canines (Jager, et.al. 2013).

In addition, it may be necessary to lengthen the time of the exercise for each horse. A treadmill or electronic walker would allow for the lengthening of the exercise time period without excess labor from the handlers involved. Lengthened exercise periods would allow for an increase in strain without an increase in speed.

The horses used in this study were in good condition but were not considered as physically fit as horses that participate in exercise regimes on a daily basis, as most jumping and endurance horses do. As previously stated they were all of good BCS and weight. However, the only exercise that they received previous to this study was daily turnout.

It is possible that the amount of exercise was simply insufficient to yield a response. Therefore, horses that were participating in studies using longer sessions at the trot, canter, and gallop would likely produce different results. Horses could also be tested at varying inclines as well.

CONCLUSION

Exercise at the level that it was conducted in this experiment was not sufficient to cause a change in the vitals, VFA, and ammonia concentrations. However, future research needs to be performed in a more controlled environment, with more robust exercise, with proper sampling to investigate the effects of exercise on the equine microbiome.

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