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Frequent riding sessions daily elevate stress, blood lactic acid, and heart rate of thoroughbred riding horses

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ABSTRACT

Minimizing stress in horses used in horse riding programs is necessary from an animal health and welfare perspective. The goal of this study was to determine the recommended horse riding frequency per day for horse riding clubs. Five Thoroughbred riding horses were rested (no riding) or ridden in 1, 2, or 4 forty-five–minute horse riding sessions per day by qualified riders for 5 weeks. For the assessment of stress, the ratio of neutrophil (N) to lymphocyte (L) was monitored 2 h after the last session. Blood lactic acid level and heart rate were determined immediately after horse riding. Blood was collected 1 day before and 17 h after horse riding for the baseline and recovery rate, respectively. The neutrophil:lymphocyte ratio, heart rate, and lactic acid levels sampled from horses ridden for four horse riding sessions were significantly higher than the levels of horses at rest. In contrast, these values for horses ridden one to two times a day showed no significant difference compared with the levels for a horse at rest. Interestingly, the frequency of bucking was significantly higher during the first horse riding session compared with that of the second or fourth horse riding sessions. It was concluded that (1) horses become more stressed when ridden four times a day than when ridden one to two times a day, and they may need a longer time to recover their health status. Furthermore, (2) horses showed a tendency of bucking during the first horse riding session after a 1-day confinement in the stable.

Introduction

Overuse can cause stress and fatigue in horses. Elevation of stress levels in horses causes abnormal behaviors, poor performance, and health problems (Odberg, 1987). Scoppetta et al. (2012) reported that prolonged exercise of horses resulted in altered plasma proteins involved in pathways for inflammation, coagulation, immune modulation, oxidant/antioxidant activity, and cellular and vascular damage. Thus, the optimal number of horse riding sessions should be evaluated to prevent overuse and subsequent stress and fatigue in horses. Several studies have been performed to test the effects of prolonged exercise on horses (Hopkins et al., 1998; Kinnunen et al., 2005; Mills et al., 1996). Most of these studies were based on the data collected from Thoroughbreds or Standardbreds exercising on a treadmill. These data are not fully applicable to a horse riding facility because of the differing environment of data collection; therefore, a study performed in the horse riding arena with riders was warranted to assess the optimal use of horses.

The definition of “prolonged exercise or overuse” can vary depending on the breed, age, sex, and condition of horses (Younes et al., 2016). The intensity of exercise (Mills et al., 1996), the level of the rider’s experience, and the horse riding environment, such as

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temperature (Janczarek et al., 2015), humidity (Mills et al., 1996), and altitude (Foreman et al., 1999) are critical factors that should be considered in the determination of what constitutes prolonged exercise levels or overuse of horses. Determinations of levels and effects of prolonged exercise should be assessed for a specific horse riding condition and a given breed of horse. In South Korea, off-track Thoroughbred horses are commonly used for horse riding lessons at commercial horse riding facilities. In general, the duration of a single horse riding lesson is approximately 45 min. Horses are normally used for one or two lessons per day. However, horse riding frequency is higher on weekends or during periods with special events. The overuse of horses cannot be prevented because there is no information or regulation regarding the maximum daily horse riding capacity of horses.

Owing to the different mechanisms and half-life, the neutrophil:lymphocyte (N:L) ratio and cortisol are used as optimal parameters to measure chronic and acute stress of horses, respectively (Park et al., 2013). The N:L ratio may respond to stressors more slowly while cortisol decreased immediately after the stressors disappeared (1-1.5 h). Thus, the N:L ratio appears to be the better parameter to measure the level of stress after the consecutive riding sessions. Lactic acid is produced and accumulated in the muscle during high-intensity exercises (Sahlin, 1986). Under conditions of high energy demand, glucose is broken down and oxidized to pyruvate. Lactate is then produced from the pyruvate faster than the body can process it, causing lactate concentrations to rise. As a result, accumulation of lactic acid is a major indicator of muscle fatigue.

Thus, the goal of this study was to determine the advisable ride frequency for horses used at horse riding facilities in Korea. Five Thoroughbred riding horses stabled at Sangju International Equestrian Center were used in this study. To monitor the status of horses, the ratio of N to L, level of lactic acid, heart rate, and horse behaviors were monitored before and after the horses had been ridden.

Materials and methods

Animals

The experiment was conducted from March to April 2016. Five Thoroughbred geldings with ages ranging from 7 to 12 years (9.2 $\pm$ 1.92 years old, 467.4 $\pm$ 18.9 kg body weight [BW]) were used in this study. The horses were stabled in 3.5 m $\times$ 3.5 m stalls with free access to an automatic water system. The stall was bedded with shavings (pine sawdust), and feces and wet shavings were removed daily. The horses were fed timothy hay three times per day (in total, 1.5% BW) and commercial concentrate three times per day (in total, 0.5% BW). The horses were housed in stalls when they were not worked.

Study design

The present study was performed consecutively for 5 weeks. On each Monday, horses were not ridden but remained stabled all day. On Tuesday, each horse was treated as follows: (1) no riding, (2) one riding session (at 3:00 pm), (3) two discontinuous riding sessions (at 11:00 am and 3:00 pm), (4) two consecutive riding sessions (at 1:00 pm and 3:00 pm), (5) four riding sessions (at 9:00 am, 11:00 am, 1:00 pm, and 3:00 pm). Each horse riding session lasted for 45 min with 5 min walking, 10 min trotting, 15 min cantering, 10 min trotting, and 5 min walking. On Tuesday of the following week, the horse was assigned to a different number of horse riding sessions (a 5 $\times$ 5 Latin square study design, Table 1). Four riders with similar riding experience rode horses to reduce the rider effect on the stress level of horses. Horses were used at various times from Wednesday to Sunday, but no data were obtained during these days.

Blood collection

Blood was collected to measure lactic acid level and to count N and L. Five to ten milliliters of jugular blood was collected using a 26-gauge needle and vacuum tube containing EDTA. For baseline data collection, blood was collected from the jugular vein at 4:00 pm on Monday (after no riding). On Tuesday, blood collection was performed at 4:00 pm and 6:00 pm (immediately after and 2 h after riding, respectively). On Wednesday, blood was also collected at 9:00 am.

Complete blood count test

To measure the stress level of horses, a complete blood count test was performed to determine the ratio of N to L in whole blood using the VetScan HM5 analyzer (Abaxis, Inc., Union City, CA). Approximately 25 μL of whole blood was transferred to a tube containing EDTA (ABAXIS, Inc.). Before analyzing the data, samples were immediately mixed by gently inverting the sample tube with potassium EDTA by hand 10 to 15 times.

Lactic acid

Blood samples were collected immediately after and 17 hours after riding. A single drop of whole blood was added in the Lactate Pro 2 test strip (ARKRAY, Inc, Kyoto, Japan), and the blood lactic acid was measured using the Lactate Pro2 instrument (ARKRAY), as per the instructions. The blood lactic acid level was measured at 4:00 pm (immediately after riding) on Tuesday and at 9:00 am on Wednesday. The baseline measurement for blood lactic acid was not performed.

Heart rate

The heart rate of horses was monitored using a stethoscope. The number of heart beats for 15 s was counted, and the number was multiplied by four to obtain the heart rate per min. For baseline data collection, the heart rate was measured at rest (4:00 pm) on Monday (no riding session). On Tuesday, the heart rate was measured at 4:00 pm (immediately after riding). On Wednesday, the heart rate was also assessed at 9:00 am.

Table 1
Horse riding schedule for 5 weeks (5 $\times$ 5 Latin Square)

Horse ID	1st week	2nd week	3rd week	4th week	5th week
1	Rest	One riding session	Two discontinuous sessions	Two consecutive sessions	Four consecutive sessions
2	Four consecutive sessions	Rest	One riding session	Two discontinuous sessions	Two consecutive sessions
3	Two consecutive sessions	Four consecutive sessions	Rest	One riding session	Two discontinuous sessions
4	Two discontinuous sessions	Two consecutive sessions	Four consecutive sessions	Rest	One riding session
5	One riding session	Two discontinuous sessions	Two consecutive sessions	Four consecutive sessions	Rest

Table 2
Ethogram for horse behavior during horse riding

	Name of behavior	Definition
1	Head down	Horses stretching their heads down
2	Head tossing	Horses stretching their heads back
3	Refusing	Horses refusing the riders' cues
4	Uncued moving forward	Horses moving forward without a rider's cue
5	Bucking	Horses jumping up and down while kicking with their hind legs

Behavior assessment

The riders and student research assistants who had experience in behavior data collection performed a qualitative behavioral assessment for each horse during the last riding session of every Tuesday starting at 3:00 pm for 5 weeks. The frequency of the behavioral events indicative of a negative mental state or distress (Hall et al., 2013), such as (1) head down (rein pulling), (2) head tossing, (3) refusing, (4) rushing (uncued moving forward), and (5) bucking was monitored (Table 2). These assessments were made and recorded by a researcher blinded to horse riding frequency treatments.

Statistical analysis

Statistical analysis of the data was performed using JMP (v. 13, SAS Institute, Cary, NC, USA). Differences in the level of stress (the ratio of N to L) and lactic acid among various frequencies of horse riding sessions and at each time point were assessed using a linear mixed model with a Tukey's post hoc analysis. Differences in horse behavior were assessed using a linear mixed model with a Tukey's post hoc analysis. Mixed models were used to account for correlation between measurements at the same point in time and correlation between measurements on the same horse. The difference was considered significant when the P-value was less than 0.05 ($P < 0.05$).

Results

The N:L ratio of horses

In the present study, the ratio of N to L in whole blood of horses was used to assess stress levels. The N:L ratio of horses measured 1 day before a horse riding session (Monday 4:00 pm after a rest, Table 3) was not significantly different among groups of horses, indicating that horses used in this study had a normal range of N:L ratios at rest. On the day of a riding session, compared with horses at rest, N:L in the whole blood collected 2 h after one riding session, two discontinuous riding sessions, and two consecutive riding sessions was not significantly different. Interestingly, however, the N:L after four consecutive riding sessions was significantly higher ($P < 0.05$) than that of horses at rest. Seventeen hours after the riding session, the N:L from horses with four consecutive riding sessions decreased, and there was no significant difference among groups.

The heart rate of horses

The heart rate was measured to monitor the condition of horses at rest or after riding. The range of heart rates for horses in normal condition is between 25 and 40 beats per min (BPM). One day before riding, the heart rate was not significantly different among groups of horses and the values were within the normal range (Table 4). After a riding session, compared with horses at rest, horses with one riding, two consecutive riding, four consecutive riding sessions exhibited an increased heart rate, but differences were not significant among these groups. At 17 h after riding, there was no significant difference in heart rates among horse groups; all were within the normal range.

Lactic acid levels of horses

The lactic acid level (mmol/L) was measured immediately after a riding session and 17 h after riding (Table 5). The level of lactic acid was not significantly different among the groups of horses with no riding, one riding session, two discontinuous riding sessions, and two consecutive riding sessions. However, the level of lactic acid in horses with four consecutive riding sessions was significantly higher ($P < 0.05$) than that of horses without riding and with one riding session.

Horse behaviors during riding sessions

Horse behaviors were monitored during the last riding session for 45 min. The frequency of head down, head tossing, refusing, rushing, and bucking was counted and recorded during the riding session (Table 6). Compared with other groups, a significantly higher frequency of bucking ($P < 0.01$) was observed in the horses during the first riding session. The frequency of other behaviors, such as head down, head tossing, refusing, and rushing in the group of horses with one riding session, was higher than that of other groups; however, these values were not statistically significant.

Discussion

In the present study, we evaluated the stress levels and physiological status (N:L ratio, lactic acid, and heart rate) of horses before and after a different number of 45 min riding sessions. The normal range of the N:L ratio for healthy horses is 0.8-2.8 (Morris and Large, 1990). The N:L ratios from horses one day before riding were within the normal range. This result indicates that horses used in this study were healthy and were not experiencing stress at this time based on this specific immunological measurement. We demonstrated that stress levels and health status of horses after one or two discontinuous or two consecutive riding sessions were similar to horses at rest. These results indicated that one or two discontinuous

Table 3
The ratio of N to L in the whole blood of horses (n = 5)

No. of riding sessions	At rest (Day 1)	2 h After riding	17 h After riding
Rest	1.84 ± 0.28	1.76 ± 0.18^{a}	1.74 ± 0.24
One	1.84 ± 0.21	1.98 ± 0.25^{a}	1.97 ± 0.36
Two discontinuous	1.66 ± 0.22	2.23 ± 0.32^{ab}	2.05 ± 0.17
Two consecutive	1.23 ± 0.24	2.33 ± 0.53^{ab}	2.42 ± 0.35
Four consecutive	2.03 ± 0.18	3.34 ± 0.52^{b}	2.31 ± 0.36

[ab]Means within a column with different superscripts indicate a significant difference at $P < 0.05$.

Table 4
The heart rate of horses (beats/min, n = 5)

No. of riding sessions	At rest (Day 1)	After riding	17 h After riding
Rest	31.00 ± 1.00	35.20 ± 1.96^{a}	33.60 ± 2.04
One	34.00 ± 1.15	40.48 ± 1.96^{bc}	35.20 ± 3.44
Two discontinuous	34.00 ± 1.15	38.40 ± 2.04^{ab}	35.20 ± 3.20
Two consecutive	33.00 ± 1.00	37.60 ± 2.40^{bc}	32.00 ± 1.26
Four consecutive	32.00 ± 2.83	44.00 ± 1.26^{c}	32.80 ± 2.33

[ab]Means within a column with different superscripts indicate a significant difference at $P < 0.05$.

Table 5
The lactic acid level of horses (mmol/L, n = 5)

No. of riding sessions	After riding	17 h After riding
Rest	0.90 ± 0.09[a]	0.76 ± 0.04
One	1.10 ± 0.06[a]	0.78 ± 0.04
Two discontinuous	0.80 ± 0.09 [ab]	0.80 ± 0.06
Two consecutive	0.90 ± 0.12 [ab]	0.76 ± 0.07
Four consecutive	1.80 ± 0.17[b]	0.84 ± 0.04

[ab]Means within a column with different superscripts indicate a significant difference at $P < 0.05$.

or two consecutive 45-min riding sessions did not cause stress or health problems for the horses. However, after four continuous riding sessions, N:L (3.34 ± 0.52) was above the normal range, indicating that the horses were stressed and they were not in normal health condition. Stress can induce the production of adrenaline and glucocorticoids via the hypothalamic–pituitary–adrenal axis with sympathetic nervous system activation (Ulrich-Lai and Herman, 2009). These hormones cause the imbalance of potent circulating inflammatory mediators, that is, interleukin-6, interleukin-1, and tumor necrosis factor-α [8] and [9] followed by the reduction of lymphocyte proliferative capacity. Thus, the elevation of N:L in the blood of horses likely provides evidence of stress or fatigue during the four consecutive riding sessions.

The blood lactic acid level also increased in horses after four consecutive riding sessions. Lactic acid accumulates in the muscles and blood during anaerobic work. In horses, aerobic work is performed when the heart rate in BPM is below 150, whereas anaerobic work is performed when BPM of horses is above 150 (Escribano et al., 2005). In addition, aerobic work is performed during walking and trotting. However, anaerobic work is performed during cantering and galloping of horses because the BPM of horses during cantering can be above 150 (Sainas et al., 2016). In this study, horses were cantering for approximately 15 min per single session, and the horses in the group with four consecutive riding sessions performed anaerobic work for approximately 1 h per day. The high level of lactic acid in the blood after four consecutive riding sessions indicates that the horses may experience a depletion of muscle glycogen (Waller and Lindinger, 2010). Glycogen in the horse muscle is produced two to three times slower than that in muscle of humans and other animals (Waller and Lindinger, 2010). Horses used for four consecutive riding sessions from 9 am to 4 pm in this study may have a depleted muscle glycogen that cannot be replaced between horse riding sessions. Thus, riding for four consecutive sessions per day is not advised because it may provide insufficient time to replace their glycogen stores between exercise sessions. Thus, horses ridden for four consecutive sessions should be allowed enough rest to recover from the heavy work load.

The heart rates of horses after one or two discontinuous or two consecutive riding sessions were within the normal range of 25-40 BPM (Mlyneková et al., 2016), although some individual horses had a higher heart rate than normal range after riding. We speculated that 5-min walking to cool down the horses before the end of the riding session may recover the heart rate to the normal range. However, horses ridden for four consecutive sessions per day showed significantly higher heart rate at the end of the last session compared with horses at rest, indicating that the cardiac recovery rate becomes lower as the duration of exercise increases. Younes et al. (2016) found similar results of decreased cardiac recovery time as exercise duration increased in young and high-endurance horses. The results of the present study also suggest that horses need more time to cool down before the end of the riding session after an extended session.

We hypothesized that horses after more frequent riding sessions should be reluctant to respond to the rider's cues because of the accumulation of stress and fatigue. However, the frequency of resistant behaviors of horses during the fourth riding session was not significantly different compared with the frequency of behaviors of horses at second discontinuous or second consecutive riding session. Interestingly, horses at the first riding session showed significantly higher frequency of bucking compared with that of the other groups. This may have occurred because before the first riding session, horses had rest in 1 day and periods of stall confinement and inactivity may result in postinhibitory rebound behavior including unwanted behavior such as bucking (Freire et al., 2009). It is interesting that although the stress level and health status were not ideal, horses at the fourth riding session did not display reluctant or avoidance behaviors. It may be because horses ridden for more sessions become more stressed and exhausted, showing fewer reluctant behaviors. One study showed that in apathetic horses, nonresponsiveness to humans' approach and interaction was reported to be a common symptom of dehydration, exhaustion, and chronic pain (Swann, 2006).

Horses used in this study had free access to water between riding sessions and they had no chronic pain. Thus, fewer reluctant behaviors were recorded from horses at the second or fourth riding session, which could have resulted from exhaustion after the riding sessions. Janczarek et al. (2015) demonstrated that the behavior of ridden recreational horses could be changed when the air temperature was above 26°C and wind speeds exceeded 5.5 m/s. In the present study, data collection was performed from March to April when the air temperature was below 26°C. In addition, the horses were ridden in an indoor horse arena, and wind was not a consideration for horse behaviors nor stress levels. Thus, horses were not exposed to possible environmental stressors that might have caused additional adverse effects on our data during the 5-week experimental period. Previous studies indicated that changes in stress levels and fatigue of horses in response to stressors may vary depending on horse conditions, such as the level of experience with riders (Ille et al., 2013), neck posture and nose position (Hall et al., 2014), equipment (Cook and Mills, 2009), and living conditions (Park et al., 2013). In this study, every horse was treated the same and they were managed under the same environmental conditions. Thus, the data collected from this study were not influenced by other stressors.

In conclusion, the four consecutive 45-min horse riding sessions is not advisable because of the elevation in the stress level and fatigue of horses. In addition, riders should be cautious during the first riding session after long-term rest because horses may show reluctant behavior. It would be wise to have experienced riders ride

Table 6
Behaviors of ridden horses (frequency, n = 5)

No. of riding sessions	Head down (rein pulling)	Head tossing	Refusing	Rushing (uncued moving forward)	Bucking
One	18.2 ± 16.5	9.8 ± 15.2	9.0 ± 15.2	3 ± 2.9	4.4 ± 3.4[a]
Two discontinuous	14.6 ± 9.8	5.2 ± 6.0	8.0 ± 6.3	1.2 ± 1.8	1.2 ± 1.1[b]
Two consecutive	11.4 ± 12.1	5.8 ± 7.0	7.2 ± 6.8	1.6 ± 2.5	1.4 ± 2.1[b]
Four consecutive	10 ± 7.4	5.2 ± 7.4	8.4 ± 11.4	2.0 ± 2.5	0.4 ± 0.5[b]

[ab]Means within a column with different superscripts indicate a significant difference at $P < 0.05$.

horses during the first riding session to acclimate these horses to riding before an unexperienced rider attempts to ride. These results should be applied to establish guidelines for use of horses with consideration of their welfare and health as well as rider safety.

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Ethical considerations

This manuscript has not been published in whole or in part elsewhere. The manuscript is not currently being considered for publication in another journal. All authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

Conflict of interest

The authors declare no conflicts of interest.

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