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Effects of ribose supplementation on selected metabolic measurements and performance in maximally exercising Thoroughbreds¹

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ABSTRACT: The objective of this study was to investigate the effects of ribose supplementation on blood ammonia-N, plasma lactic acid, plasma glucose, volume of oxygen consumption (VO₂), heart rate, and performance in Thoroughbred geldings performing a maximal treadmill standardized exercise test (SET). The hypothesis tested was that ribose supplementation would decrease ammonia-N and lactic acid accumulation during exercise, and improve performance. Eight Thoroughbred geldings were assigned randomly to one of two groups: glucose or ribose. The glucose group received 0.15 g glucose/kg of BW, and the ribose group received 0.15 g of ribose/kg BW top-dressed on the feed twice daily. After 2 wk of glucose or ribose supplementation, a SET was performed. Blood was analyzed for blood ammonia-N, plasma lactic acid, and plasma glucose before exercise (0 min), every minute during SET, and at

15 and 30 min after exercise. Heart rate and VO₂ were recorded for the duration of SET. After a 10-d washout period, geldings switched groups. Following another 2 wk of supplementation, a second SET was performed, and same data recorded. Blood ammonia-N and plasma lactic acid increased as duration of SET increased and reached a peak at 15 min after exercise. Peak plasma glucose was observed at 15 min after exercise, and peak heart rate and VO₂ were recorded at highest speed during SET. Geldings supplemented with ribose had blood ammonia-N, plasma lactic acid, plasma glucose, VO₂, heart rate, and performance similar to those of geldings supplemented with glucose. Results from this study show that supplementation with 0.15 g ribose/kg BW twice daily in the diet of conditioned Thoroughbred geldings for 2 wk does not influence blood ammonia-N, plasma lactic acid, plasma glucose, VO₂, heart rate, or performance during SET or the first 30 min of recovery.

Key Words: Exercise, Horse, Lactic Acid, Performance, Ribose, Thoroughbred

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Introduction

Exhaustive exercise causes muscle ATP levels to significantly drop below resting values (Cheetman et al., 1986; Schuback and Essen-Gustavsson, 1998; Schuback et al., 2000) because ATP is utilized at a higher rate than the maximum capacity of the muscle to rephosphorylate ADP. To maintain an adequate ATP:ADP ratio, ATP is generated via the adenylate kinase reaction by combining two molecules of ADP to form a molecule of ATP and a molecule of adenosine monophosphate (AMP). During maximal exercise, AMP can be further deaminated to inosine monophosphate (IMP; Harris et al., 1987; Essen-Gustavsson et al., 1997; Schuback et al., 2000). Inosine monophos-

phate can then be reaminated back to AMP via the purine nucleotide cycle. However, some IMP is catabolized to inosine, hypoxanthine, and xanthine, which can diffuse out of the myocyte, resulting in a net loss of total adenine nucleotide (TAN) pool (Braut and Terjung, 2001). Total adenine nucleotide pool is replenished through the slow de novo synthesis (Sheehan and Tully, 1983). Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme during the production of 5-phosphoribosyl-1-pyrophosphate (PRPP) via the pentose phosphate pathway (Eggleston and Krebs, 1974; Kliezien et al., 1994; Tian et al., 1998). Further, PRPP is the limiting factor in adenine nucleotide synthesis (Zimmer, 1980; Zimmer and Ibel, 1983; Boer and Sperling, 1995). Reports indicate that pentoses (i.e., ribose) and pentiols can bypass the rate-limiting step by contributing to the formation of PRPP (Segal and Foley, 1958; Hauschildt and Watts, 1976; Zimmer, 1998), which can then be utilized to replenish TAN. Recent studies indicate that ribose supplementation in men increases power output in sprint sessions (Raue et al., 2001) and results in significant increases in muscular

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Table 1. Composition and nutrient analysis of concentrate and hay

Ingredient (as-fed basis)	Concentrate, %	Coastal bermudagrass hay
Cracked corn	34.25	—
Crimped oats	26.50	—
Soybean meal, 48% CP	10.00	—
Wheat bran	10.00	—
Blackstrap molasses	8.00	—
Alfalfa meal pellets, 17% CP	7.50	—
Ground limestone	1.50	—
Dicalcium phosphate	0.80	—
Sodium chloride	0.75	—
Vitamin premix ^a	0.30	—
Vitamin E ^b	0.15	—
Lysine 98%	0.05	—
Luprosil (mold inhibitor) ^c	0.10	—
Trace mineral premix ^d	0.10	—
Nutrient, DM basis ^e		
Digestible energy, Mcal/kg	3.3	2.1
Crude protein, g/kg	162.9	94.7
Acid detergent fiber, g/kg	137.0	358.4
Neutral detergent fiber, g/kg	333.2	771.1
Ether extract, g/kg	33.4	10.1
Calcium, g/kg	10.8	3.1
Phosphorus, g/kg	7.3	1.9

^aProvided 4,400 kIU vitamin A, 440 kIU vitamin D, and 35,200 IU vitamin E/kg premix.

^bProvided 44,200 IU vitamin E (DL- α -tocopherol acetate)/kg premix.

^cBASF Corp., Mount Olive, NJ.

^dProvided 14,300 mg Cu, 40,000 mg Zn, 28,000 mg Fe, 28,000 mg Mn, 80 mg Co, 160 mg I, and 160 mg Se/kg premix.

^eDigestible energy was calculated from NRC (1989); other nutrient values were based on laboratory analysis.

to 8 m/s, and the treadmill surface elevated to a 6° inclination. Geldings exercised for 1 min at each of the speeds 8, 9, 10, 11, 12, and 13 m/s until they failed to stay in place on the treadmill despite urging. The treadmill was then lowered to a flat surface and the geldings were taken off the treadmill and hand-walked for 30 min. Blood samples were collected before exercise (0 min), during the last 20 s of each exercise step (8, 9, 10, 11, 12, and 13 m/s), and at 15 and 30 min postexercise. Blood for ammonia-N determination was collected

in sodium heparin tubes (Becton Dickinson, Franklin Lakes, NJ), and immediately deproteinized using 10% sodium tungstate (wt/vol) and 1 N sulfuric acid (McCullough, 1967). The supernatants were frozen at -80°C until ammonia-N was determined, within 24 h after sample collection, by the procedure described by McCullough (1967). Blood for plasma lactic acid and glucose was collected in potassium oxalate/sodium fluoride tubes (Becton Dickinson), and centrifuged within 30 min after collection. Plasma was collected and frozen at -80°C until analyzed for lactic acid (#735-10) and glucose (#315-500) by colorimetric procedures (Sigma, St Louis, MO). Volume of oxygen (VO_2) intake (Columbus Instruments, Columbus, OH) and heart rate (Hewlett-Packard, Palo Alto, CA) were recorded for the duration of each SET. Due to difficulties with the equipment, VO_2 results include data from only seven geldings. Performance was recorded as the total run time in seconds beginning at 8 m/s and continued until the geldings could no longer stay in place on the treadmill despite urging. After a 10-d washout period, geldings switched groups. During the washout period, geldings continued to be stabled in their stalls, fed with the same schedule, and exercised four times per week. After washout and another 2 wk of supplementation, a second SET was performed, and the same data recorded.

Statistical Analysis

Data are presented as mean \pm SEM. Data were analyzed by a mixed model for crossover, with repeated measures using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The model included the main terms of horse, time, period, treatment, and treatment \times time interaction. Effects were considered significant at $P < 0.05$.

Results

Blood ammonia-N (Table 3) increased as the duration of the SET increased, and reached a peak at 15 min postexercise in each group ($P < 0.001$). Geldings supple-

Table 2. Body weight, BCS, and concentrate and hay intake of eight geldings during training and supplementation periods

Time, wk	Body weight, kg	Body condition score ^a	Concentrate intake, kg ^{bc}	Hay intake, kg ^{bc}
1	513.3 \pm 16.3	4.4 \pm 0.3	3.1 \pm 0.1	2.6 \pm 0.1
3	525.6 \pm 16.6	4.5 \pm 0.3	3.3 \pm 0.1	2.6 \pm 0.1
5	520.0 \pm 12.7	4.6 \pm 0.2	3.3 \pm 0.1	2.6 \pm 0.1
7	519.8 \pm 13.1	4.5 \pm 0.1	3.4 \pm 0.1	2.6 \pm 0.1
9	519.3 \pm 12.6	4.6 \pm 0.1	3.4 \pm 0.1	2.6 \pm 0.1
11	524.8 \pm 12.8	4.7 \pm 0.1	3.4 \pm 0.1	2.6 \pm 0.1
13	520.9 \pm 12.6	4.7 \pm 0.1	3.4 \pm 0.1	2.6 \pm 0.1
15	522.9 \pm 12.7	4.7 \pm 0.1	3.4 \pm 0.1	2.6 \pm 0.1

^a1 = poor, 2 = very thin, 3 = thin, 4 = moderately thin, 5 = moderate, 6 = moderately fleshy, 7 = fleshy, 8 = fat, 9 = extremely fat.

^bConcentrate and hay amounts shown were fed twice per day at 0800 and 1700.

^cAs-fed basis.

Table 3. Blood ammonia-N ($\mu\text{mol/L}$) of eight geldings supplemented for 2 wk with either 0.15 g/kg BW glucose or ribose before, during, and after a standardized exercise test

Item	Glucose	Ribose	SEM	<i>P</i> -value ^a
Before exercise, 0 min	35.7 ^b	33.6 ⁱ	4.7	0.65
8 m/s	40.5 ^{bc}	39.9 ^j	4.2	0.85
9 m/s	43.7 ^c	41.0 ^{jk}	3.6	0.08
10 m/s	53.4 ^d	47.6 ^k	5.2	0.28
11 m/s	106.4 ^e	75.6 ^l	20.6	0.28
12 m/s	132.6 ^f	205.5 ^m	39.8	0.42
15 min after exercise	334.3 ^g	322.5 ⁿ	35.4	0.81
30 min after exercise	258.0 ^h	233.1 ^o	30.9	0.57

^a*P*-value for differences between glucose and ribose in the same row (time \times treatment); mixed model (*P* < 0.001).

^{b,c,d,e,f,g,h,i,j,k,l,m,n,o}Values with different superscripts within the same column differ (*P* < 0.05).

mented with ribose had blood ammonia-N similar to that of geldings supplemented with glucose at similar sampling times. Peak blood ammonia-N was 334.3 ± 35.4 and 322.5 ± 35.4 $\mu\text{mol/L}$ in glucose and ribose groups, respectively (*P* = 0.81, Table 3).

Plasma lactic acid (Table 4) increased as the duration of the SET increased and reached a peak at 15 min postexercise in each group (*P* = 0.001). Peak plasma lactic acid was 26.5 ± 1.1 and 26.2 ± 1.1 mmol/L in glucose and ribose groups, respectively (*P* = 0.83). Geldings supplemented with ribose had lower (*P* = 0.03) plasma lactic acid before exercise (0 min) than geldings supplemented with glucose. At all other times, geldings supplemented with ribose had plasma lactic acid similar to that of geldings supplemented with glucose (Table 4).

Geldings supplemented with ribose had plasma glucose (Table 5) similar to that of geldings supplemented with glucose. Peak plasma glucose occurred at 15 min postexercise and was 6.96 ± 0.22 and 7.18 ± 0.22 mmol/L in glucose and ribose groups, respectively (*P* = 0.43; Table 5).

After ribose supplementation, four geldings increased their total run time, three geldings decreased their total run time, and one gelding did not change its total run time compared to glucose supplementation (data not shown). Geldings supplemented with ribose

had similar total run time as geldings supplemented with glucose (279.4 ± 9.9 vs. 272.5 ± 9.9 s, *P* = 0.58). Geldings supplemented with ribose had heart rates (Table 6) and VO_2 (Table 7) similar to those of geldings supplemented with glucose at all sampling times.

Discussion

The racehorse is often limited by its ability to maintain the speed necessary to win races and to recover after strenuous exercise so that it is ready for the next competition. Previous research indicates that the decreased availability of ATP contributes to the loss of contractile properties by skeletal muscle. Researchers have shown that ATP is almost depleted in certain equine muscle fibers after intense track or treadmill exercise, and it takes several days to regenerate the ATP used during exercise (Snow et al., 1985; Schuback and Essen-Gustavsson, 1998; Schuback et al., 2000). Recently, dietary supplementation with ribose was proposed to enhance energy recovery by inducing increases in ATP concentrations, which are associated with increased contractile performance, reduced cell damage, and ultimately delaying the onset of muscle fatigue.

In a previous study performed in our laboratory (Kavazis et al., 2002), daily ribose supplementation (0.07 g/kg BW twice daily) for 14 d in the diet of exercising

Table 4. Plasma lactic acid (mmol/L) of eight geldings supplemented for 2 wk with either 0.15 g/kg BW glucose or ribose before, during, and after a standardized exercise test

Item	Glucose	Ribose	SEM	<i>P</i> -value ^a
Before exercise, 0 min	0.7 ^b	0.6 ^k	0.03	0.03
8 m/s	5.0 ^c	5.3 ^l	0.6	0.66
9 m/s	9.0 ^d	9.2 ^m	0.8	0.88
10 m/s	13.2 ^e	12.6 ⁿ	1.2	0.71
11 m/s	19.4 ^{fg}	17.6 ^{op}	1.6	0.41
12 m/s	22.1 ^h	24.1 ^q	1.6	0.49
15 min after exercise	26.5 ⁱ	26.2 ^r	1.1	0.83
30 min after exercise	20.7 ^{gi}	19.6 ^{ps}	1.3	0.55

^a*P*-value for differences between glucose and ribose in the same row (time \times treatment); mixed model (*P* < 0.001).

^{b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s}Values with different superscripts within the same column differ (*P* < 0.05).

Table 5. Plasma glucose (mmol/L) of eight geldings supplemented for 2 wk with either 0.15 g/kg BW glucose or ribose before, during, and after a standardized exercise test

Item	Glucose	Ribose	SEM	<i>P</i> -value ^a
Before exercise, 0 min	4.97 ^b	5.01 ^g	0.16	0.83
8 m/s	4.76 ^{bc}	4.84 ^g	0.11	0.47
9 m/s	4.69 ^{cd}	4.80 ^g	0.11	0.29
10 m/s	4.81 ^{bd}	4.95 ^g	0.13	0.31
11 m/s	4.94 ^b	4.99 ^g	0.15	0.79
12 m/s	4.98 ^b	5.23 ^g	0.19	0.41
15 min after exercise	6.96 ^e	7.18 ^h	0.22	0.43
30 min after exercise	6.31 ^f	6.69 ⁱ	0.23	0.22

^a*P*-value for differences between glucose and ribose in the same row (time × treatment); mixed model (*P* < 0.001).

^{b,c,d,e,f,g,h,i}Values with different superscripts within the same column differ (*P* < 0.05).

geldings resulted in lower blood ammonia-N and plasma lactic acid during recovery after SET. However, a single dose of ribose (250 g of ribose dissolved in 3 L of water given via a nasogastric tube 1 h before SET) did not result in any metabolic differences. Therefore, this study was designed to supplement horses with a daily dose for 14 d because results from previous study indicated that daily oral ribose supplementation may have been beneficial to exercising Thoroughbred geldings. In the current study, the amount of ribose given to geldings was doubled (0.15 g/kg BW twice daily) so that the amount being used (supplement-to-BW ratio) was comparable to the amount of ribose used in human studies that reported ergogenic effects of daily oral ribose supplementation (Raue et al., 2001; Van Gammeren et al., 2002). Despite the differences observed in the previous study by Kavazis et al. (2002) for blood ammonia-N and plasma lactic acid, there were no differences between groups for these two metabolites in the current study.

Dietary supplementation with ribose was proposed to enhance energy recovery by inducing increases in ATP concentrations, which are associated with increased contractile performance, reduced cell damage, and ultimately a delay in the onset of muscle fatigue. Several *in vitro* studies have shown that ribose infusion into isolated skeletal muscle increases the recovery of ATP. Tullson and Terjung (1991) reported that ribose,

administered to isolated hind limb muscle fibers, led to a 3.4- to 4.3-fold increase in adenine nucleotide de novo synthesis rates. Brault and Terjung (2001) reported that ribose significantly increased the adenine salvage rate in hind limb muscle, and eliminated the decrease in adenine salvage rate that was observed with the control. Zarzeczny et al. (2001) reported that ribose enhanced the formation of PRPP, the precursor to the synthesis of TAN, thereby increasing ATP synthesis. Recently, researchers have attempted to document the effects of oral ribose supplementation as an ergogenic aid in exercising humans, but the results do not provide a clear and definitive answer. Raue et al. (2001) reported that daily ribose supplementation in men significantly increased power output in the last of a series of sprint sessions and increased mean power over a 5-d training period when compared to glucose. Van Gammeren et al. (2002) reported that daily ribose supplementation resulted in significant increases in muscular strength and total work performed in recreational bodybuilders. However, Op'T Eijnde et al. (2001) reported that ribose supplementation had no beneficial impact on postexercise muscle ATP recovery or maximal intermittent exercise performance in men. Berardi and Ziegenfuss (2003) reported that ribose supplementation did not have a substantial effect on anaerobic cycle sprinting in men.

Table 6. Heart rate (beats/min) of eight geldings supplemented for 2 wk with either 0.15 g/kg BW glucose or ribose during a standardized exercise test

Item	Glucose	Ribose	SEM	<i>P</i> -value ^a
8 m/s	195 ^b	199 ^e	5	0.52
9 m/s	203 ^b	207 ^e	3	0.33
10 m/s	212 ^c	213 ^f	2	0.65
11 m/s	217 ^d	218 ^g	2	0.86
12 m/s	220 ^d	221 ^h	2	0.68

^a*P*-value for differences between glucose and ribose in the same row (time × treatment); mixed model (*P* < 0.001).

^{b,c,d,e,f,g,h}Values with different superscripts within the same column differ (*P* < 0.05).

Table 7. Volume of oxygen consumption (VO₂; mL·kg⁻¹·min⁻¹) of seven geldings supplemented for 2 wk with either 0.15 g/kg BW glucose or ribose during a standardized exercise test

Item	Glucose	Ribose	SEM	<i>P</i> -value ^a
8 m/s	63 ^b	68 ^f	9	0.71
9 m/s	114 ^c	114 ^g	4	0.83
10 m/s	137 ^d	137 ^h	4	0.89
11 m/s	149 ^e	146 ⁱ	4	0.14
12 m/s	150 ^e	150 ⁱ	4	1.00

^a*P*-value for differences between glucose and ribose in the same row (time × treatment); mixed model (*P* < 0.001).

^{b,c,d,e,f,g,h,i}Values with different superscripts within the same column differ (*P* < 0.05).

High-intensity exercise produces ammonia, and the accumulation of ammonia indicates that ATP is being produced via the adenylate kinase reaction ($2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$) to supply the ATP needed during exercise. Adenine monophosphate is subsequently being deaminated to IMP and ammonia, which indicates a failure of the metabolic pathways to keep pace with the metabolic production of ATP. Sewell and Harris (1992) reported a high correlation between IMP and ammonia and that ammonia is the preferable marker of adenine nucleotide degradation as compared with other adenine nucleotide catabolites (Rasamen et al., 1993; Harris et al., 1999). In the present study, blood ammonia was similar between the glucose and ribose groups, indicating that ribose may not have been used to increase ATP concentrations and decrease the rate of the adenylate kinase reaction.

Plasma lactic acid is highly correlated with IMP (Sewell and Harris, 1992). In the present study, plasma lactic acid was similar between the two groups, which may indicate that ribose had no effect on adenine nucleotide metabolism. Plasma glucose concentrations did not differ between groups, indicating that ribose supplementation did not cause changes in glucose kinetics under the conditions of this experiment. Performance was similar between the two groups, indicating that ribose supplementation may not be beneficial for maximally exercising geldings under the conditions of this study.

Heart rates increased as the speed of the treadmill increased. Highest heart rates were recorded during the last exercise step, and heart rates observed in this study were similar to heart rates reported by other researchers for horses performing similar SET (Hodgson and Rose, 1994; Marlin and Nankervis, 2002). The increase in VO_2 , heart rate, blood ammonia, and plasma lactic acid concentrations in the current study indicate that the exercise protocol induced marked anaerobic responses. Peak blood ammonia and plasma lactic acid concentrations were detected at 15 min postexercise. At 30 min postexercise, ammonia and lactic acid concentrations decreased compared with 15 min after exercise but were higher than the samples taken before exercise. Differences might have been detected between groups if blood samples had been taken for a longer time after the end of the SET.

The hypothesis investigated in this study was that oral ribose supplementation could be used as an ergogenic aid during high-intensity exercise. Ribose supplementation, in the diet of conditioned Thoroughbred horses, did not benefit the horses during a SET under the conditions of this experiment. Based on these results, ribose supplementation did not show that it can be used as an effective ergogenic aid for exercising Thoroughbred geldings.

Implications

The results from this study show that supplementation with 0.15 g of ribose per kilogram of body weight

twice daily in the diet of conditioned Thoroughbred geldings for 2 wk did not influence blood ammonia-nitrogen, plasma lactic acid, plasma glucose, volume of oxygen consumption, heart rate, or performance during a standardized exercise test or during the first 30 min of recovery. In conclusion, oral ribose supplementation did not affect anaerobic exercise capacity or metabolic markers in trained geldings as evaluated by this protocol.

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