

Ergogenic Evaluation of Exercise Performance in Response to Ingestion of H2Plus Technologies Water in Horses

Prepared and submitted by

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Introduction

“H2Plus Technologies water” is a proprietarily re-structured form of traditional water manufactured by H2Plus Technologies, Inc. H2Plus Technologies claims to alter the physical-chemical properties of traditional water to match that of intracellular water found in living cells. In doing so the cells do not have to expend the energy to convert it to the observed finished structure of intracellular water. The cells can then repurpose that restructuring energy to further growth, performance, endurance, reduce recovery time and accelerate intracellular healing. The ingestion of H2Plus Technologies water is claimed to have beneficial effects on the performance or productivity and wellness of animals, including horses. Recently, the maximal rate of ATP synthesis in equine mitochondria incubated in H2Plus Technologies-produced water was shown to be significantly increased by 25% when compared to the rate of synthesis associated with incubation in standard ultrafiltered laboratory water. The next step in the process of investigating effects of H2Plus Technologies water in horses was to extend its evaluation from ex-vivo to an in-vivo study.

Objective

The aim of this study was to assess the effect of saturation of body water with H2Plus Technologies water on exercise performance in fit Thoroughbreds completing: (1) a stepwise treadmill exercise test to determine maximum oxygen consumption (VO_2max) and, (2) a run to fatigue at a fixed submaximal exercise intensity ($\text{VO}_2 = 75 - 80\% \text{VO}_2\text{max}$). The point of fatigue was deemed to have been reached when horses could no longer maintain the speed of the treadmill despite verbal encouragement.

Experimental Design

1. Two unfit horses participated in a pilot water safety evaluation for 21 days with blood samples for complete blood counts and equine plasma ion and biochemistry panels being collected before, and 10 and 21 days after they started drinking the H2Plus Technologies water. Their daily water intake was also monitored. This safety evaluation occurred while the other horses in the study were undergoing their initial training.
2. Following completion of the water safety assessment, a randomized crossover study was performed in which 8 fit Thoroughbred horses were randomly assigned to one of two groups. Horses in Group A initially drank re-structured water provided by H2Plus Technologies for 18-21 days prior to undergoing the first of two sets of treadmill tests. Following the completion of the first set of treadmill tests, their water source was switched to that provided by the local water supply and they drank that for at least 18 days before repeating the same set of treadmill tests. Based on mathematical models of water turnover, this duration of H2Plus Technologies water ingestion should result in >90% of body water being H2Plus Technologies water.
3. Group B horses drank water sourced from the local supply until after completing the first set of treadmill tests. Their drinking water was then switched to that provided by H2Plus Technologies 25 days before they were scheduled to repeat the treadmill tests.
4. All horses received a standard diet of grass hay and a sweet feed-based concentrate twice daily. They were conditioned on the WSU Hitchcock Research Racetrack for a minimum of 7 weeks before performing any treadmill tests. During this period, they were also be acclimated to running on the WSU high speed treadmill. The racetrack conditioning program was akin to that used by professional racehorse trainers in preparing Thoroughbreds to race. Following completion of the first set of



treadmill tests, the horses were ridden regularly in order to maintain the same level of fitness throughout the period between the first and second sets of treadmill tests.

Each set of treadmill tests consisted of 2 different assessments of performance. For all treadmill tests, the treadmill incline was maintained at 10%. The first assessment measured maximal aerobic capacity (VO₂max). This was a stepwise test in which the treadmill speed increased in 1 m/s increments every minute until there was no increase in VO₂ despite an increase in speed, or until the horse was unable to maintain the speed of the treadmill. The second assessment tested the horse's aerobic endurance by measuring the time at which it ran at the treadmill speed calculated to have an oxygen requirement that was 75 - 80% of the VO₂max until it was unable to maintain its position on the treadmill. Blood for measurement of post-exercise lactate concentration was taken 2 minutes after finishing the run to fatigue.

The VO₂max values, blood lactate concentrations and run times to fatigue were compared statistically using a 1-way repeated measures analysis of variance to test for effects of drinking the H2Plus Technologies water as compared to Pullman tap water. Differences were regarded as significant when $p \leq 0.05$.

Results

Safety data

The results are summarized in Table 1. There was no sign of any hematological or serum biochemical abnormality associated with the consumption of H2Plus Technologies water for 21 days. The horses' water consumption was also normal and ranged from 10 – 16 gallons/day.

Table 1

Variable	Horse 1			Horse 2			Normal Range
	day 0	day 10	day 21	day 0	day 10	day 21	
White blood cells	11,800	8,400	8,500	8,200	8,000	6,400	5.5-10.5 x10 ³
PCV	50	46	44	42	48	37	30-45
Hemoglobin	18.7	17.1	16.4	15.2	17.3	13.7	11-16
Total Protein	6.4	6.7	6.2	6.5	6.4	6.0	5.6-7.5
SDH	4	2	2	1	1	1	4-14
GGT	25	26	22	35	27	20	14-40
AST	270	308	315	242	272	225	170-435
CK	394	282	257	223	585	215	30-357
BUN	18	18	16	19	18	17	10-27
Creatinine	1.1	0.8	0.8	1.1	1.0	0.9	0.8-1.9
Glucose	100	78	91	59	91	90	47-110
Albumin	4.2	4.2	3.9	4.2	4.2	3.7	2.8-4.1
Globulin	2.2	2.5	2.3	2.3	2.2	2.3	1.8-4.1
Calcium	12.3	12.9	12.0	12.0	12.9	11.8	10.7-14.1
Phosphate	3.0	3.5	3.3	3.0	2.8	2.8	2.2-4.7
Bilirubin	1.4	1.2	1.3	2.0	1.4	1.3	0.6-2.9
Sodium	146	143	141	140.0	140	143	132-141
Potassium	2.3	4.1	3.7	4.0	4.3	3.1	2.4-5.6



Chloride	105	104	102	100.0	104	104	96-106
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Maximal Oxygen Consumption (VO_{2max})

The data from one horse had to be deleted due to a technical error during one of the treadmill runs. For $n=7$, the p value approached significance ($p = 0.06$, Fig 1A)). This could be interpreted as meaning that there was a 94% certainty that drinking H2Plus Technologies increased VO_{2max} . It was also notable that 4 of the horses were able to run at 12 m/s (26.8 mph at 8% incline) after drinking the H2Plus Technologies water (H) while only one of the control (tap water, (T)) horses reached this speed, and could only maintain it for 20 sec (Fig 1B). The speed at which VO_{2max} was reached however, was not different for the two treadmill tests (9.9 ± 0.7 m/s (T) vs 9.8 ± 1.1 m/s (H); $p = 0.79$). This suggested that the percentage of total energy being produced by aerobic means was higher following H2Plus Technologies ingestion than after drinking tap water, given the higher VO_{2max} at these points. This is of relevance in terms of performance because the lower the anaerobic contribution to total energy production, the longer the horse can run at its VO_{2max} all other things remaining the same. Or, put another way, the further or longer it can run at a given speed until fatigue sets in.

Fig 1A

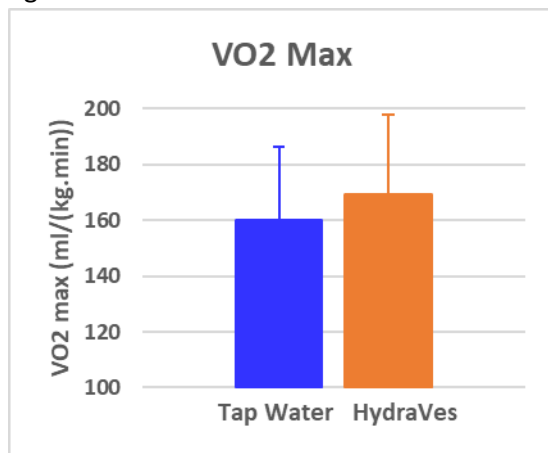
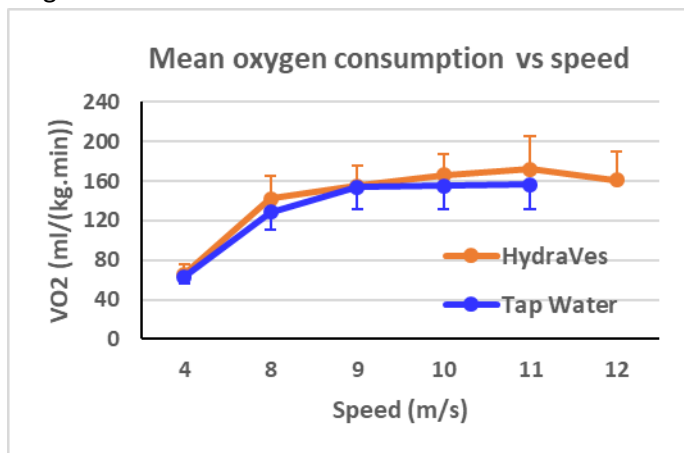


Fig 1B



Run Time to Fatigue

Seven of the 8 horses ran longer after drinking H2Plus Technologies. Three horses failed to run for 15 mins in the control run and one of them also failed to run 15 mins after drinking H2Plus Technologies water although the latter run was about 2min45s longer. All other horses ran >15min after drinking H2Plus Technologies. The difference in the runs time to fatigue approached significance ($p = 0.07$). One horse ran longer in the control (T) run (there was a cooler ambient temperature that day) and if its data are deleted ($n = 7$, $p = 0.02$), the horses ran more than 2 mins longer with H2Plus Technologies (908 ± 182 sec (T); 1047 ± 232 sec (H); Fig 2B).

The relative intensity at which these tests were completed did not differ ($77.8 \pm 13.2\%$ VO_{2max} (T) vs $77.3 \pm 9.9\%$ VO_{2max} (H), $p = 0.99$). The fact that these intensities were not different between the two tests was reassuring, as it was important that they completed each test at the same intensity as the most supportable conclusion is that the same horses displayed more stamina after drinking H2Plus Technologies.



Fig 2A (n=5 for T vs n=7 for H2Plus Technologies at 15 mins)

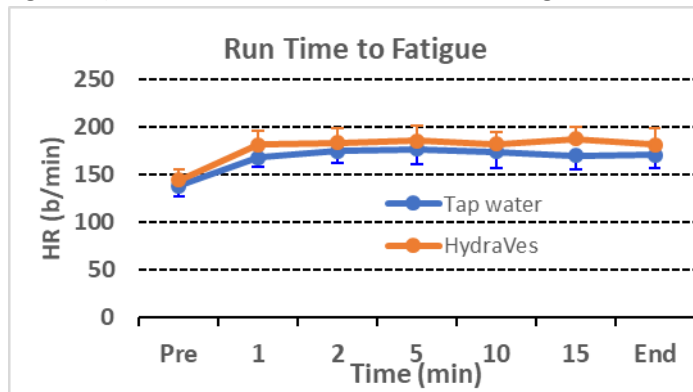
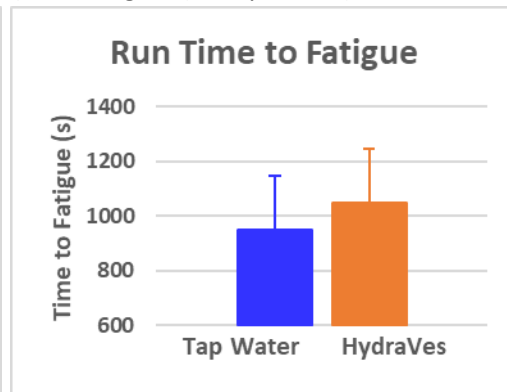


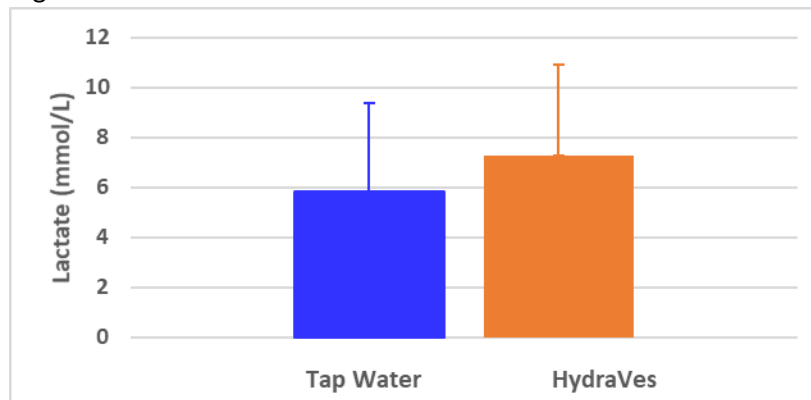
Fig 2B (n=7, p = 0.02)



Lactate

There was no difference in post-exercise blood lactate concentration (5.9 ± 3.5 mmol/l (T) vs 7.3 ± 3.6 mmol/l (H), $p = 0.09$, Fig 3) although there was a tendency for the value to be higher after the run associated with consumption of H2Plus Technologies water. This was not surprising given the difference in the run times to fatigue and the kinetics of lactate production. Anaerobic energy production is not an “on-off” situation. Anaerobiosis is reflected by the blood lactate concentration and is always present at some level regardless of the level of exercise or lack of it. As a rule, the longer a horse or person runs at a moderate ($>60\%$ VO_{2max}) to high ($>90\%$ VO_{2max}), the higher the lactate concentration as this is compatible with our finding.

Fig 3



Conclusions

The horses' health was not adversely affected by drinking the H2Plus Technologies water. There were no detectable changes in their blood profiles and there was no change in their daily water consumption. The horses were fit when they began the study and there was no apparent improvement or decrease in their aerobic capacity over the course of the study. Despite this, their VO_{2max} was increased (94% probability) and their endurance was enhanced as reflected by the marked increase in the run time to fatigue in 7 of the 8 horses. These findings are compatible with and affirm the ex-vivo finding that mitochondrial ATP synthesis was enhanced in the presence of H2Plus Technologies water. The reason for the improvement in stamina also may be that the saturation of the body with re-structured H2Plus Technologies water may have been associated with a reduction in the presence and/or effect of oxidants



like reactive oxygen species (ROS) in the muscles. Reduced production of ROS in the long head of the triceps following H2Plus Technologies water consumption was mentioned by Dr Michael Davis in his January report on the muscle biopsy component of this investigation and that is definitely compatible with an increase in the efficiency of ATP production due to the finished structure of H2Plus Technologies water representing that of intracellular water.

