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Final report

Does the energy we are fueling the horses actually break the engine?

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Part 1: Detailed summary

Syftet med studien var att undersöka effekten av olika nivåer av stärkelseintag på muskelenzymaktivitet i plasma efter intensiv travträning. Ökade nivåer av muskelenzymaktivitet i plasma är en indikation på läckage från muskelceller och/eller muskelsönderfall. I studien togs blodprover och mättes hjärtfrekvens i samband med snabbjobb på 76 varmblodiga travhästar tränade av 5 olika professionella travtränare. Hästarna var två till 12 år gamla och i den provtagna gruppen ingick 12 hingstar, 28 ston och 36 valacker. Under snabbjobben hade hästarna genomsnittlig maxhjärtfrekvens på 215 slag per minut. Genomsnittlig mjölksyrakoncentration i plasman var 3,1 mmol/l (0,5 till 34 mmol/L). Blodproven analyserades för muskelenzymaktivitet (kreatininkinas (CK) och aspartat aminotransferas (AST)) i plasma samt plasmakoncentrationen av acetat, glukos, insulin och mjölksyra. Resultaten visar på högre muskelenzymaktivitet efter träning samt högre muskelenzymaktivitet, framförallt AST, hos de hästar som åt mer än 450 gram stärkelse per dag än hos de som åt mindre än 268 g stärkelse per dag. De hästar som utfodrades med mindre än 268 g stärkelse per dag hade högre nivåer av fettsyran acetat i blodet efter träning än de som utfodrades med mer stärkelse. Resultaten visade också att stona i studien utfodrats med lägre mängder stärkelse än hingstar och valacker. Trots detta hade stona högre muskelenzymaktivitet i plasma än hingstar och valacker.
Resultaten visar på att tillgängligheten av fettsyran acetat är högre hos hästar som får lägre andel kraftfoder och högre andel grovfoder i sin foderstat. Vilket gör att hästar med högre andel grovfoder har mer acetat att använda som energi under träning. Att ston utfodras med lägre mängder kraftfoder kan bero på att ston har en högre risk att drabbas av korsförlamning. Högre nivåer av muskelenzymaktivitet i plasma efter träning hos de hästar som åt höga nivåer av stärkelse tyder på att muskelsönderfall går att minska med lägre stärkelsegivor. Det behövs dock ytterligare studier att förstå muskelsönderfall och läckage av muskelenzymer i samband med intensiv träning.

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En av effekterna av intensiv träning är olika nivåer av muskelsönderfall vilket är en del av det som ger ett träningsframsteg. Hos kapplöpningshästar, där återhämtningen mellan träningspass är kort, skulle en utfodringsstrategi som minimerar muskelsönderfall vara intressant.

Part 2: Main report (max. 10 pages)

Introduction

The feed offered to athletic horses must be energy-dense to meet their high-energy requirements. Traditionally, this requirement has been met by large amounts of high-energy concentrates rich in starch. A review of feeding practices for racehorses in the USA, Germany, Australia, and Sweden reported concentrate allowances of 6.8±0.4 kg/day and forage allowances of 5.8 ±0.4 kg/day (Jansson & Harris, 2013). However, there are problems connected to feeding high amounts of concentrate (starch-rich diet) as increased risk of rhabdomyolysis (McLeay *et al*., 2000), colic (Tinker et al., 1997; Hudson et al., 2001), gastric ulcers (Luthersson et al., 2009), and stereotypic behaviors (Redbo et al., 1998). To decrease the risks associated with feeding large amounts of concentrate, the diet can be adjusted to contain more or only high-energy forage. That a forage-only diet can be an alternative for athletic horses has been shown in earlier studies (Connysson et al., 2017; Jansson & Lindberg, 2012). These forage-only diets are not only preventing health issues, but they have also been shown to be beneficial for some performance parameters like the lactate threshold (V_{La4}, Jansson & Lindberg, 2012).

Elevated muscle enzyme activity concentrations (creatinine kinase (CK) and aspartate aminotransferase and (AST)) in plasma are always connected to leakage from damaged muscle. These leakages from the muscle can be indications of delayed onset muscle soreness (DOMS), muscle injury, or muscle diseases like recurrent exertional rhabdomyolysis (Hogeson, 2014). A study from 2010 (Isgren et al., 2010) indicates that 6% of the trained Standardbred trotters in Sweden are affected by the rhabdomyolysis syndrome. In the study of Isgren et al. (2010), the horses were fed 4.5 kg (range 3–6 kg) of concentrate and 9 kg (range 6–11 kg) of forage.

Exercise intensity and energy substrate availability are two of the factors that influence substrate use. At lower intensities (30 % of $V0_{2max}$), glucose, glycogen, and lipids support approximately 10%, 30%, and 60%, respectively, of the energy expenditure, and at more intense exercise (60% Vo_{2max}) these values are ~5%, 70%, and 25%, respectively (Geor et al 2000). Availability of energy substrate e.g. increased concentrations of free fatty acids during exercise results in a greater increased energy supply from fatty acid oxidation (Rennie et al., 1976). In humans, an increased free fatty acid concentration has been found to increase fat oxidation at 85% VO_{2max} (Romijn et al., 1995). Increased fat availability has also been shown to alter energy substrate oxidation during low-intensity exercise in horses (Pagan et al., 2002). Compared with grain-based (starch) diets, forage diets increase the concentration of the volatile fatty acid acetate in plasma (Connysson et al., 2017; Jansson & Lindberg, 2012) and thereby increase the availability of fatty acids.

Problem: It is generally accepted that muscle enzyme activity (CK and AST) levels in plasma may increase in Standardbreds and Thoroughbreds after exercise and racing (Kristensen et al., 2017, Lindholm 1987, Pösö et al., 1983, Siciliano et al. 1995), indicating muscle damage and/or increased leakage. However, in one recent study on healthy Standardbred trotters fed a forage-

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only diet muscle enzyme activity levels were very low after race-like exercise tests (Connysson et al. 2021). Interestingly, lowered muscle enzyme responses after exercise have also been observed in horses with recurrent exertional rhabdomyolysis (MacLeay et al., 1999; McKenzie et al., 2003), as well as in healthy athletic horses (MacLeay et al., 1999), when fed low starch diets.

Aim: The study aimed to evaluate the effect of different levels of starch intakes on plasma muscle enzyme activity post-exercise.

Hypothesis: The hypothesis was that the plasma muscle enzyme activity levels considered normal for athletic horses are highly dependent on starch-rich diets and to a lesser degree dependent on exercise.

Material and methods

This study was performed during September-January 2022-2023. Seventy-six horses from five professional trainers (licensed by the Swedish Trotting Association) were included (7-37 horses at each trainer). The trainers had different feeding strategies and fed different amounts of concentrates to their horses. Information on sex and age was collected from the Swedish Harness Racing Association. The horses were 2-12 years old. There were 12 colts, 28 fillies, and 36 geldings. The exercise was performed as interval exercise, up-hill interval exercise, heat exercise, or race.

Sampling

Blood samples were collected at rest, before exercise (R) and within 25 minutes after (A) the horses had performed their ordinary intense exercise sessions (after the last heat/interval). The mean time from the last heat/interval to sampling was 15 minutes (range 7-25; SE 0.5 minutes). During exercise, the horses had an average maximal heart rate of 215 (SE 1.3) beats per minute (bpm) ranging from individual max heart rate registrations of 172 to 237 bpm. The average duration of exercise with a heart rate over 200 bmp was 258 (SE 19.9) seconds with a range of 0 to 722 seconds. The average plasma lactate concentration post-exercise was 3.1 (SE 0.6) mmol/L with the range 0.5 to 34 mmol/L (enzymatic and spectrophotometric method Boehringer Mannheim/R-Biopharm, Darmstadt, Germany).

Diet

Information on feed allowances was collected for each horse. The trainers used different commercially available concentrates and a sample from each concentrate was collected and analyzed for starch content. The mean starch content of the concentrates was 286 (SE 31.6) g/kg feed ranging from 153-467 g/kg feed. The total starch intake was then calculated from feed allowances and starch content. Some horses had different concentrate intakes on different days, and for those horses, a mean daily starch intake was estimated. The horses were divided into three groups based on daily starch intake (low, medium, and high, see Table 1). One horse was in the medium group on the first occasion and in the high group on the second occasion and is therefore included in both groups.

Table 1. *Starch intake groups, range of starch intakes in each group, and number of horses in each group.*

Diet group	Daily starch intake (g)	Number of horses
Low	0-268	18
Medium	451-967	26
High	988-1429	33

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Measurements and analyses
Blood samples were collected by venipuncture from the jugular vein in 6-mL lithium-heparinized tubes (102 IU) and kept on ice until centrifuged (10 min, 920 x g,), after which the plasma was frozen (-20°C).

Plasma acetate concentration, glucose concentration, AST activity, and CK activity were analyzed in all samples. The plasma activity of AST, CK, and concentrations of glucose was analyzed with Beckman Coulter DxC 700AU automatic biochemistry analyzer with reagents from Beckman Coulter (land). Plasma acetate concentrations were analyzed with an enzymatic and spectrophotometric method (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany). Plasma lactate concentrations were only analyzed in the sample taken after exercise (A). Plasma insulin concentration was only analyzed in samples taken at rest (R) using ELISA 2 (Mercodia equine insulin kit, Mercodia, Uppsala, Sweden).

Heart rate was continuously recorded during exercise using a heart rate recorder (Polar M460 Polar Electro, Kempele, Finland) and the data were analyzed using the program Polar Flow (Polar Electro, Kempele, Finland).

Statistical analysis
Analysis of variance was performed with the PROC MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Statistical analysis of CK, AST, glucose, and acetate was performed with a model including fixed effects of age class, sex, occasion, starch intake group, exercise group, sample, and the interaction between starch intake group and sample. The horses were divided into three age classes (class 1: 2 years old; class 2: 3-4 years old, class 3: 5-12 years old). The model for an observed variable of horse (trainer) i, age class j, sex k, occasion l, starch intake group m, exercise group n, sample o was:

$$Y_{ijklmno} = \mu + \eta_i + \pi_j + \gamma_k + t_l + \chi_m + h_n + \lambda_o + (\lambda h)_{mo} + e_{ijklmno}$$

where μ is the overall mean, η_i is the effect of horse, π_j is the effect of age class, γ_k is the effect of sex, t_l is the effect of occasion, χ_m is the effect of starch intake group, h_n is the effect of exercise group, λ_o is the effect of sample $(s\lambda)_{mo}$ is the effect of the interaction between starch intake group and sample, and $e_{ijklmno}$ is the random error. The random part included horse (trainer) and trainer. Observations within each horse (trainer) * trainer were modeled as repeated measurements.

For insulin, a statistical analysis was performed with a model including fixed effects of age class, sex, occasion, and starch intake group. The random part included horse. Observations within each horse were modeled as repeated measurements.

For starch allowance analysis of variance was performed with the PROC GLM procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) with the effects of age class and sex and the interaction of age class and sex. In these analyses, only the horses fed concentrates were included (n=64).

Results and discussion

Results
Plasma AST activity was higher after exercise than before on all starch intakes (Table 2). Plasma AST activity was significantly lower on low starch intake than on medium and high

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starch intake both before and after exercise (Table 2). Plasma CK activity was higher after exercise than before in horses fed medium starch intake and higher after exercise on medium starch intake compared to low and high starch intake (Table 2).

Plasma glucose concentrations were higher after exercise than before on all starch intakes (Table 2), but there were no differences in glucose concentrations between starch intake groups. Plasma acetate concentrations were lower after exercise than before in horses with high starch intakes. There was a tendency for plasma acetate concentrations to be higher after exercise than before on the low starch diet. Before exercise, plasma acetate concentrations were higher in horses with high starch intakes and low starch intakes than in horses with medium starch intakes. After exercise, plasma acetate concentrations were higher in horses with low starch intakes than in horses with high and medium starch intakes (Table 2).

Horses on high starch intake had higher plasma insulin concentrations than horses on medium starch intake and a tendency of higher plasma insulin concentrations than horses on low starch intake (Table 2).

Table 2. *Plasma activity of creatine kinase (CK), aspartate aminotransferase (AST), and plasma concentrations of glucose, acetate, and insulin in trained Standardbred trotters on three different starch allowances*

Variable	**Diet**	**Before exercise**	**After exercise**	**SE**	**P-value (before vs. after exercise)**
Plasma AST (ukat/L)	High	7.8[a]	8.1[a]	0.5	<0.0001
	Medium	7.9[a]	8.5[a]	0.5	<0.0001
	Low	5.7[b]	6.1[b]	0.8	<0.0001
Plasma CK (ukat/L)	High	4.2	4.5[a]	0.5	0.531
	Medium	3.8	6.0[b]	0.4	<0.0001
	Low	3.1	3.9[a]	0.5	0.115
Plasma glucose (mmol/L)	High	5.7	6.4	0.2	0.006
	Medium	5.5	6.0	0.2	0.017
	Low	5.4	6.1	0.2	0.008
Plasma acetate (mmol/L)	High	0.78[a]	0.67[a]	0.04	0.002
	Medium	0.67[b]	0.70[a]	0.04	0.423
	Low	0.87[a]	0.93[b]	0.05	0.090
Plasma insulin (mU/L)	High	21.7[a]	-	2.2	-
	Medium	14.2[b]	-	2.4	-
	Low	15.3[c]	-	3.0	-

[a,b] *Different letters in the same column = significant ($p<0.05$) difference between starch intakes.*
[a,c] *Different letters in the same column = tendency ($p<0.10$) for the difference between starch intakes.*

Effects of sex

Plasma AST activity and plasma CK activity was higher for fillies (F) than colts (C) and geldings (G) ((F:8.59 ukat/L (SE 0.52); C: 6.58 ukat/L (SE 0.82); G: 6.46 ukat/L (SE 0.48)

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(P<0.04)) and plasma CK activity ((F:4.92 ukat/L (SE 0.26); C: 3.71 ukat/L (SE 0.42); G: 3.94 ukat/L (SE 0.24) (P<0.01)). There was no significant difference between fillies and colts on plasma glucose concentration, but fillies had higher plasma glucose concentration than geldings (P=0.02) ((F: 5.98 mmol/L (SE 0.16); C: 5.65 mmol/L (SE 0.24); G: 5.56 mmol/L (SE 0.15)). There was no significant difference between sex on plasma acetate concentrations (M: 0.71 mmol/L (SE 0.03); C: 0.81 mmol/L (SE 0.05); G: 0.74 mmol/l (SE 0.03)) or plasma insulin concentrations (F: 15.03 mU/L (SE 2.11); C: 20.05 mU/L (SE 3.52); G: 16.10 mU/L (SE 1.92)). In addition starch allowance was lower to fillies (771 g/day SE 66) than colts and geldings (1024 g/day (SE 101) and 1003 g/day (SE 59), respectively).

Discussion

The results from this study indicate that both high-intensity exercise and starch intake affect plasma CK and AST activity in healthy-trained Standardbred trotters. Increased muscle enzyme activity after intense exercise/racing has been shown in many earlier studies (Kristensen et al., 2017, Lindholm 1987, Pösö et al., 1983, Siciliano et al. 1995). Muscle enzyme activity in the present study is relatively low and could reflect that blood samples were collected relatively short (7-25 minutes) after exercise. CK reaches peak concentrations within a few hours after exercise, and AST reaches peak concentrations after approximately 24 hours (Harris et al., 1998). Since this was a field study performed on privately owned horses during normal exercise the number of blood samples taken had to be restricted and the possibility to control sampling time was limited. The limited possibility to control sampling time was also reflected in the low average plasma lactate concentrations, which probably was due to the cool-down jogging of the horses 7-25 minutes before blood sampling. The disappearance of blood lactate has been shown to increase if the horse is active during short-term recovery (Marlin et al., 1987).

Lowered plasma muscle enzyme activity in response to low/no starch intake is in accordance with earlier findings during controlled studies in healthy horses (Connysson & Jansson, 2022; MacLeay et al., 1999) and in horses with recurrent exertional rhabdomyolysis (MacLeay el al., 1999; McKenzie et al., 2003). The lower plasma CK values in the high and medium starch intake groups in the present study compared to the concentrate diet group in Connysson & Jansson (2022) (4.6 and 5.9 vs. 12.2 ukat/L) might be explained by higher starch intakes in Connysson & Jansson (2022) than in the present study (1673-3100 g starch/day vs. 451-1429 g starch/day). The elevated AST in the present study and Connysson & Jansson (2022) could indicate that plasma AST is elevated from the exercise session before the sampled one. Most Standardbred trotters have an exercise program with rest 72-96 hours between exercise sessions. Plasma AST has a half-life of 7-10 days (Cardinet et al., 1967).

Since elevated muscle enzyme activity in plasma is an indicator of muscle damage and/or increased leakage it could be speculated why starch intake affects the level of this leakage. In the present study, the metabolic parameters differed between the different starch intake groups indicating that energy substrates available during exercise were different in the different groups. The main difference was higher acetate values in the horses on low/no starch intakes which is in accordance with earlier studies on forage-only/mainly forage diets (Connysson et al., 2017; Jansson and Lindberg, 2012). Acetate is produced during fermentation of fibers in the hindgut and is taken up by most cells in the body and is converted by acetyl-CoA synthetases to acetyl-CoA and metabolized via the citric acid cycle. The muscle cells' selection for fuels during exercise depends on exercise intensity but also the availability of different energy substrates (Hodgson, 2014). Both Jansson &Lindberg (2012) and Martin et al. (2023) have shown that no-starch diets alter metabolic response in trotters

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during exercise probably due to more available acetate and less energy contribution from the glycolysis. In addition, no-starch diets have been shown to change energy metabolism during exercise and give a slower response in lactate accumulation (Jansson &Lindberg, 2012; Palmgren-Karlsson et al., 2010).

In the present study, there is no clear connection between starch intake and plasma insulin concentration since there is no difference between medium and low starch intake groups. However, the high starch intake group has higher insulin concentrations than the groups with lower starch intakes. This lack of difference is probably because the horses were fed at different time points before blood sampling e.g. horses were fed in the morning and the blood samples was collected approximately 0.5-4 hours after feeding in connection to exercise. Higher insulin concentration levels in horses fed starch than in horses feed no/low starch diets have been shown earlier (Pagan et al., 1987; Willams et al., 2001; Connysson et al. 2010; Jansson & Lindberg, 2012; Jensen et al.; 2016). In Jansson and Lindberg (2012) starch-rich diets resulted in elevated insulin levels in Standardbred trotters during warm-up and recovery in connection to sub-maximal exercise. In the present study, there was no effect of starch intake on plasma glucose concentrations. This can also be due to the blood sampling timing in relation to feeding since plasma glucose concentrations are affected by differences in the timing of feeding in relation to exercise (Duren et al., 1999; Pagan & Harris, 1999). Elevated plasma glucose concentrations after exercise are due to an exercise-induced increase in cortisol levels. Cortisol increases glucose concentration in the blood.

That fillies had higher plasma muscle enzyme activity than colts and geldings is in accordance with earlier studies on two and three-year-old Thoroughbred racehorses (Harris et al., 1990; Harris et al., 1998). In the present study, the higher muscle enzyme activity in fillies was even though a lower mean starch intake in the fillies. In addition, fillies have an increased risk of developing exertional rhabdomyolysis (Harris, 1991; Mc Gowan et al., 2002). In humans, higher CK levels in males than females after training are reported and estrogen has been suggested to be an important factor in this sex effect that might be explained by estrogen's membrane stabilizing effect that reduces the intracellular calcium inflow (Oosthuyse & Bosch, 2017).

Conclusions

This study showed higher muscle enzyme activity of AST and some elevations of CK activity after exercise and in horses fed high-starch intakes compared to low-/no-starch intakes. This indicates that starch intake and energy substrate metabolism affect muscle damage in healthy Standardbred trotters.

Relevance for the practical horse sector incl. recommendations

Intense exercise induces different levels of muscle damage. These damages can cause myofibrillar disruption, swelling, efflux of myocellular enzymes and myoglobin, and also an inflammation process in the muscle that leads to adaptive remodeling of muscle (Peak et al., 2017). There is, however, no common well-defined definition for measuring degrees of exercise-induced muscular damage. In athletes, as racehorses, where recovery between exercises is short it would have been interesting to decrease muscle damage. The present study indicates that one strategy to do that could be to alter energy metabolism with diets low in starch.

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Part 3: Result dissemination

State all result dissemination from the financed project into the appropriate section, including information as indicated in each section. Additional rows can be added to the table.

Scientific publications, *published*	Connysson, Malin, och Anna Jansson. ”Starch Allowance and Muscle Enzyme Activity in Healthy Standardbred Trotters Trained by Professional Trainers”. *Journal of Animal Physiology and Animal Nutrition* Åtkomstdatum 08 maj 2025. https://doi.org/10.1111/jpn.14127.
Scientific publications, *submitted*	
Scientific publications, *manuscript*	
Conference publications/ presentations	M. Connysson, G. Öst, and A. Jansson, *“Starch allowance in trained Standardbred trotters”, EWEN 2024, Oslo.* *https://www.europeanworkshoponequinenutrition.org/ewen-2024*
	Connysson M. & Jansson A. “*Field study: plasma aspartate aminotransferase (AST) activity in healthy Standardbred trotters fed different starch allowances” EEHNC 2025, Utrecht*
Other publications, *media etc.*	Hästsverige : https://hastsverige.se/news/forskning-mindre-kraftfoder-minskar-risken-for-muskelsonderfall-hos-hast/
	SLU: https://www.slu.se/forskning/kunskapsbanken/vh/sport--och-sallskapsdjur/hast/mangden-kraftfoder-verkar-oka-lackaget-av-muskelenzym-hos-hastar-i-traning/
	Reportage i travronden under kommande veckor. Intervjuad av Josefine Johansson 250618
Oral communication, *to horse sector, students etc.*	Förhandsföreläsning på proffstränarkursen VT 2024
	Förhandsföreläsning Hästforskarträff SLU HT 2024
	Föreläsning proffstränarkursen VT 25
	Föreläsning träningsfysiologiutbildning * 3 (ca 90 deltagare) VT 2025 (Hagmyren, Solvalla, Färjestad)
Student theses	*Öst, G., 2023. (supervisor M. Connysson) Stärkelseintagets inverkan på muskelenzymaktivitet hos varmblodiga travhästar. Master thesis.* *https://stud.epsilon.slu.se/19512/1/Öst_Greta_230918.pdf*

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Other	

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