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Effect of dietary energy source on the plasma parameters of equine athletes trained in a deep water aqua treadmill

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ABSTRACT

The aim of the study was to test the effect of different dietary energy sources on several blood biochemical parameters on aqua treadmill trained show jumpers. Four horses in latin square arrangements consumed identical amounts of meadow hay, and four concentrates differing mainly in their energy source (control, starch from oat, oil from sunflower and sugar from sugar beet molasses) but providing the same amount of energy. One experimental period consisted of a 10 day adaptation and 4 day test period involving deep water aqua treadmill training. Blood samples were taken during and after the last aqua treadmill training and lactate, lactate dehydrogenase, creatine kinase, aspartate aminotransferase, glucose and triglycerides levels were determined from the plasma. The different dietary energy sources resulted in similar plasma lactate levels. The increased starch content of the feed resulted in significantly lower ($p < 0.05$) creatine kinase level at the end of the first walking section of aqua training. This result appeared later as a tendency ($p \leq 0.1$). Horses fed sunflower oil as a main energy source had higher aspartate aminotransferase level after two hours of the aqua training. The plasma triglyceride concentration in the sunflower oil group tended ($p < 0.1$) to be lower at the end of aqua training; while one hour after the training it was significantly lower. The elevated level of creatine kinase and aspartate aminotransferase indicates that lactate does not correctly reflect the strenuousness of the aqua training. The dietary energy source modifies the metabolic response to aqua training, even if it is not considerable.

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Introduction

A proper energy supply has a primary importance for the equine athlete (Pagan 1998). The source of energy has an influence on health, metabolism and sport performance (Harris 2009). Therefore, the preference of energy sources depends on the type, intensity and length of the workload. Several publications demonstrate the effect of carbohydrates and fats as energy sources on various blood parameters in horses (Pagan & Jackson 1995; Pagan et al. 1995; Spangfors 1998; O'Connor et al. 2001; Treiber et al. 2008). Research results show that the training of horses in water could be a good alternative or supplement to conventional training (Nankervis et al. 2009; Lindner et al. 2010). However, it is also demonstrated that the cooling effect of water markedly alters the metabolic response of horses to aqua training measured by various plasma biochemical parameters (Hevesi et al. 2009; Lindner et al. 2012). Thus, it can be hypothesised that the plasma biochemical response altered by different dietary energy sources when deep water aqua exercise is part of the training program. The daily rations of equine athletes should include a mixture of energy sources (starch, fat, fibre) in a balance (Pagan 1998). Any extremes (e.g. unbalanced energy supply) should be avoided. Therefore, the aim of this study was to examine the effect of different main dietary energy sources on several blood biochemical parameters on high level aqua treadmill trained show jumpers using the energy source more diffused under field conditions.

Materials and methods

Experimental animals

Four normally trained show jumpers aged from 6 to 11 years were used in the test at the Pannon Equestrian Academy, Kaposvár University, Hungary. Gender was not considered in the selection of the animals tested.

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Table 1. Feed allowance and composition of treatment groups (kg).

	Treatments			
Feed component[a]	Control	Starch	Total sugar	SF Oil
Muesli[b]	0.25	0.20	–	–
Pelleted oats	1.25	2.05	0.50	0.50
Supplements[c]	1.10	0.20	2.00	0.80
Molasses (beet)	–	–	0.30	–
Sunflower oil	–	–	–	0.40
Meadow hay	12.0	12.0	12.0	12.0

[a]Concentrate components were mixed and served as three equal meals at 6:00. 12:00 and 17:00. Hay was provided in two equal portion in the morning and evening feeds.
[b]Heim Tier Land GmbH & Co KG, Happy Horse Sensitive Kräuter
[c]Heim Tier Land GmbH & Co KG, Happy Horse Basic Vollwert Pellet

Table 2. Daily nutrient intake of the treatment groups with hay and concentrate.

		Concentrate (according to treatments)			
Nutrient	Roughage[a] Meadow hay	Control	Starch	Sugar	SF Oil
Dry matter, kg	11.0	2.3	2.2	2.5	1.6
Crude protein, g	696	346	330	320	155
Crude fat, g	264	114	120	95	453
Crude fibre, g	3192	275	224	297	145
Starch, g	0.0	911	1076	698	423
Total sugar, g	900	166	100	345	88
DE[b], MJ	103.6	30.9	30.9	30.7	30.5

[a]Identical amount in case of each treatment
[b]DE calculated according to Zeyner and Kienzle (2002)

Treatments

Four dietary treatments were formulated and applied in a Latin square design. The horses consumed an identical amount of meadow hay, but four daily concentrate portions were formulated (Table 1) to provide different main energy sources but an identical amount of digestible energy (Table 2). The control group received the concentrate normally fed in the structure, while the three other concentrates provided an elevated level of starch, total sugar and fat, respectively. The daily nutrient supply was sufficient or in excess to a horse with medium exercise intensity (National Research Council 2007). Water and salt blocks were freely available to the horses. No variance in salt consumption was noticed. One experimental period consisted of a 10 day adaptation and 4 day test period involving deep water aqua treadmill training. The relatively small difference between dietary treatments made it possible to change the diets without a transition period.

Training program

The horses were trained according to the schedule presented in Table 3. Normal training was one hour with a rider, while jumping training was half an hour warming up and half an hour jumping with a rider. The protocol of the 44 minute long aqua training and blood sampling is reported in Table 4. During the aqua treadmill training the temperature of the water was 21 °C, while the level of the water was set to 85% of the height at the withers. The temperature of the water was kept in reserve tanks on 21 °C constantly with a circular heating system to perform the same protocol. This program lasted 44 minutes and after the training the horses were dried under infra-red lamps for 20 minutes. The horses were then taken back to the stable.

Table 3. Training program of the 14 day experimental periods.

	Days													
	Sat 1	Sun 2	Mon 3	Tue 4	Wed 5	Thr 6	Fri 7	Sat 8	Sun 9	Mon 10	Tue 11	Wed 12	Thr 13	Fri 14
Normal training	X		X	X		X		X		X	X	X		
Jump. training					X		X						X	
Aqua training											X	X		X

Table 4. Schedule of the aqua training and blood sampling.

Phase	Time (min)	Speed of aquatrainer (km/h)	Blood sampling, min (code)	Activity
0	0	–	0 (T0)	Standing, preparation
1	0–10	4.5	10 (T1)	Walking, filling up the aquatrainer
2	10–40	13.0	40 (T2)	Trot in water
3	40–44	4.5	44 (T3)	Walking, emptying the aquatrainer
4	44–60	–	60 (T4)	Standing under infrared lamps
5	60–120	–	120 (T5)	Resting in the box
6	120–180	–	180 (T6)	Resting in the box

Blood sampling

4 ml blood samples were taken during day 14 of the aqua treadmill training program at the time indicated in Table 4. These samples were taken from the jugular vein via catheters and placed in sampling tubes containing NaF-oxalate or Na-heparine. The blood samples were stored on ice until centrifugation. The samples were centrifuged at 3000 rpm for 3 minutes. Plasma was pipetted to an Eppendorf tube and stored at a temperature of −18 °C until the analysis.

Laboratory analysis

All feed components used in the trial was sampled and analysed for crude protein (93/28/EEC), crude fibre (92/89/EEC), crude fat (98/64/EC), total sugar (71/250/EEC) and starch (99/79/EC) content. The DE content of feed components was calculated according to the

equation of Zeyner and Kienzle (2002). From the blood plasma samples lactate, lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), glucose and triglyceride levels were determined using the Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring system.

Statistical analysis

The experimental data were evaluated by the SAS 9.1 (SAS Institute Inc., Cary, NC) statistical software package using the GLM procedure. The blood parameter values measured at rest (before exercise – T0) were used as a covariate in the course of the statistical analyses. In case of significant treatment effect mean differences were tested by a Ducan's multiple range test.

Results and discussion

The measured lactate values (Figure 1) suggest that the energy requirement of aqua treadmill training was ensured by an aerobic energy supply, since the measured values were below the generally accepted anaerobic threshold of 2 mmol/l (Eaton 1994). Training with higher speed (19.8 km/h) but with similar water level (80% of the height of the withers) resulted in similar lactate levels (Lindner et al. 2012) compared to our observations. Lower water levels at the same treadmill speed results in higher lactate values (Lindner et al. 2010). Similar to other studies (Hevesi et al. 2009; Vincze et al. 2012), plasma lactate levels did not increase during the high level aqua treadmill training, but only after the workout. Studies have shown that the temperature of the water can alter the heart beat (Nankervis et al. 2009), and according to these results, based on the evolution of lactate levels, it can be concluded that the lower temperature of the water (21 °C) below that of body temperature reduces the production of lactic acid through anaerobic glycolysis by reducing the temperature of the muscles. The thermoneutral zone for horses has not been established yet (Lindner et al. 2012), but in humans, this value ranges from 33 to 35 °C (Choukroun & Varene 1990). These values are likely to be lower in horses (Nankervis et al. 2009), but most probably higher than the temperatures we applied. These results suggest that the lactate response depends not only on the speed of the treadmill, but also on the level of the water as well.

The dietary fat content can be utilised only in the aerobic energy yielding processes, while the starch can be used in both an aerobic and anaerobic way (Pagan 1998). The training program failed to induce the need for an anaerobic energy supply, and this could also explain the lack of differences in plasma lactate level between fat and starch treatments (Figure 1). A similar conclusion was reached using corn and fish oil supplementation (O'Connor et al. 2001). Ultimately, it seems that horses around the age of 7 years and having good fitness express a high activity of lactate clearance to prevent the formation of significant peaks of this metabolite in plasma. This is supported by the elevated lactate dehydrogenase level (647–732 U/L; data not presented) compared to the reference range (162-412 U/L; Kaneko et al. 2008) and the fact that neither

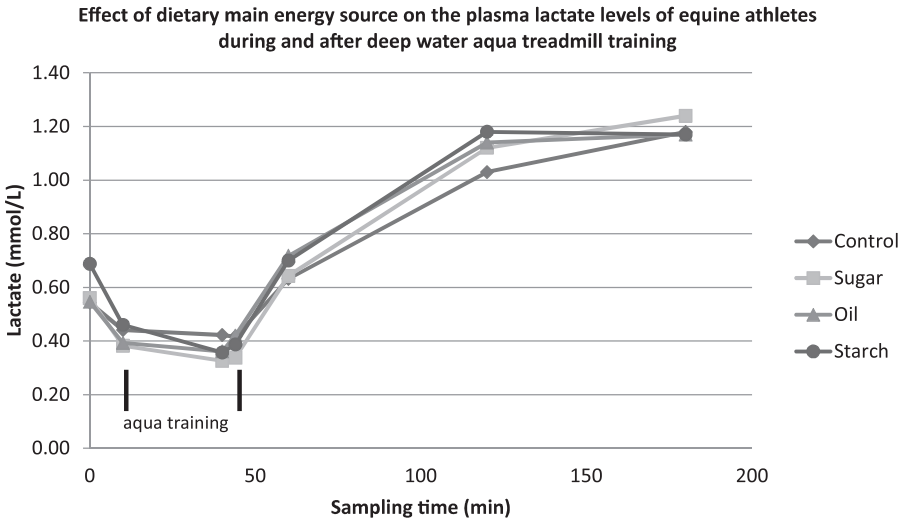

Figure 1. Effect of dietary main energy source on the plasma lactate levels of equine athletes during and after deep water aqua treadmill training

Table 5. Effect of dietary main energy source on the plasma creatine kinase levels (U/L) of equine athletes during and after deep water aqua treadmill training.

Gait	Stand (preparation)	Walk (water)	Trot (water)	Walk (water)	Stand (under infrared)	Stand (in the box)	Stand (in the box)
Sampling time, min	0 (T0)	10 (T1)	40 (T2)	44 (T3)	60 (T4)	120 (T5)	180 (T6)
Control	208	216[a]	206	193	196	218	210
Sugar	207	223[a]	219	216	219	217	204
Oil	229	221[a]	214	201	205	231	226
Starch	195	181[b]	187	176	185	190	189
P_{model}	0.66	<0.001	<0.001	<0.001	0.0020	<0.001	<0.001
P_{T0}		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$P_{treatment}$	0.66	0.002	0.20	0.06	0.31	0.09	0.10
RMSE	38.1	12.8	20.7	18.0	24.5	20.9	18.6

RMSE: root mean square error.
[a,b]Mean values within a column with similar superscript letters are not significantly different ($p > 0.05$).

treatments nor aqua training had a significant effect on the LDH development.

The increased starch content of the feed resulted in a significantly lower ($p = 0.002$) creatine kinase level at the 10th minute of high level aqua training (at the end of the first walking section) (Table 5). This result appeared as a tendency ($p \leq 0.1$) at the 44, 120 and 180 minutes sampling as well. Various reference values exist for CK: 11–130 U/L (Lumsden et al. 1980) and 90–270 U/L (Southwood 2013). The values we found fall into the upper range, and no exercise induced increase can be observed (Table 5). Interestingly, significantly lower CK values were measured in show jumpers performing in higher class competitions (Art et al. 1990a,1990b). Other experimental results also indicate that excessive training does not result in an increase of CK activity (Harris et al. 1997; Hamlin et al. 2002). Moreover, the show jumping test failed to further increase elevated CK level due to muscle biopsy (Soares et al. 2013). Pritchard et al. (2009) established a reference value of 210 U/L for working horses in Lahore (Pakistan). According to their explanation, the relatively high value is probably the result of low-level but chronic muscle injuries (caused by the actual everyday work done by these horses), and not a reversible result of a single exhaustive exercise bout. These observations indicate that horses subject to regular but relatively short intensive exercises could have chronic muscle damage which results in a somewhat elevated CK level (about 200–300 U/L). Only prolonged endurance exercise (60 km or more) can result in very high levels of CK (1000–30 000 U/L) (Kerr & Snow 1983; Volfinger et al. 1994; Adamu et al. 2013). The increase of CK activity can be the result of muscle cell damage or increased cell membrane permeability. Only CK activity higher than 10 000 U/L presents some evidence of myolysis (Volfinger et al. 1994). Therefore, in our case, the increased muscle cell membrane permeability caused the elevated CK activity. It can be concluded, that the intensity of the training program (including deep water aqua training) was high enough to achieve that increase in cell membrane permeability. The explanation for differences in reference values (Lumsden et al. 1980; Southwood 2013) can be the different training level of the subjects tested.

Horses having sunflower oil as a main energy source in their concentrate had higher aspartate aminotransferase enzyme level after two hours of the aqua training (Table 6). This difference appears as a tendency at the end of the walking sections as well. The measured levels of AST fall within the wide range of reference values set for sport horses (Kaneko et al. 2008; Lumsden et al. 1980; Southwood 2013). Significantly lower AST values were observed in higher class show jumping horses (Art et al. 1990a,1990b) compared to our results. However, in English Thoroughbred horses with good racing results continuously elevated (around 300 U/L) AST values were found (Harris et al. 1990). Oliveira et al. (2014) measured AST levels well above 300 U/L in eventing horses tested on treadmill. Most of our measured AST values are close to the 300 U/L activity. As also AST mainly released from muscle, the simultaneously elevated AST and CK levels indicate a strenuous exercise. Horses fed a starch enriched diet expressed lower CK at T2 and AST at T6; and tended to express lower CK at T3, T5, T6 and AST at T1, T3 sampling compared to other treatments. These results suggest that starch as an energy source may improve the muscles' ability to cope with strenuous exercise, although the physiological background is still unclear.

The dietary treatments resulted in similar plasma glucose concentrations (Table 7). The measured concentrations were within the reference values for horses (Kaneko et al. 2008; Southwood 2013). Experimental results have demonstrated that when carbohydrates are substituted with fat (oil) on isocaloric bases, the blood glucose and insulin levels decrease (Pagan et al. 1995; Stull et al. 1987). Lower glucose levels were

Table 6. Effect of dietary main energy source on the plasma aspartate amino transferase levels (U/L) of equine athletes during and after deep water aqua treadmill training.

Gait	Stand (preparation)	Walk (water)	Trot (water)	Walk (water)	Stand (under infrared)	Stand (in the box)	Stand (in the box)
Sampling time, min	0 (T0)	10 (T1)	40 (T2)	44 (T3)	60 (T4)	120 (T5)	180 (T6)
Control	274	277	272	266	262	280	274[a,b]
Sugar	272	277	277	280	274	283	275[a,b]
Oil	291	284	282	278	281	283	289[a]
Starch	262	257	264	260	265	265	260[b]
P_{model}	0.70	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{T0}		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$P_{treatment}$	0.70	0.08	0.39	0.07	0.11	0.13	0.02
RMSE	34.1	13.6	14.1	10.8	11.4	11.4	10.4

RMSE: root mean square error.
[a,b]Mean values within a column with similar superscript letters are not significantly different ($p > 0.05$).

Table 7. Effect of dietary main energy source on the plasma glucose levels (mmol/L) of equine athletes during and after deep water aqua treadmill training.

Gait	Stand (preparation)	Walk (water)	Trot (water)	Walk (water)	Stand (under infrared)	Stand (in the box)	Stand (in the box)
Sampling time, min	0 (T0)	10 (T1)	40 (T2)	44 (T3)	60 (T4)	120 (T5)	180 (T6)
Control	4.4	3.7	3.7	4.2	4.3	5.1	5.7
Sugar	4.4	4.1	4.0	4.1	4.1	5.0	5.6
Oil	4.3	3.9	3.8	3.8	4.5	4.9	5.3
Starch	4.8	3.9	4.0	3.9	4.3	5.0	5.6
P_{model}	0.60	0.43	0.30	0.25	0.08	0.66	0.96
P_{T0}		0.18	0.05	0.05	0.01	0.15	0.79
$P_{treatment}$	0.60	0.58	0.88	0.65	0.71	0.99	0.91
RMSE	0.53	0.41	0.58	0.53	0.45	0.67	0.91

RMSE: root mean square error.

observed after dry treadmill training of Thoroughbreds when 15% of the daily energy intake was provided as oil (Crandell et al. 1998). In our experiment, horses in the sunflower oil treatment group received about 11.5% of their daily energy intake as vegetable oil. The difference between the two energy supply levels as oil is not considerable, so it does not justify the lack of effect. Therefore most likely the cooling effect of water is responsible for that metabolic response. When oil substitutes soluble carbohydrates (starch, sugar) in the feed the adaptation processes reduce the glucose substrate dependence of the work (Treiber et al. 2008). This mechanism slows down the depletion of glycogen stores during long and strenuous work, preventing the development of metabolic dysfunction such as insulin resistance. The quality (fatty acid composition) of the dietary fat source also modifies the glucose metabolism. Fish oil supplementation resulted in lower glucose levels compared to the corn oil fed group (O'Connor et al. 2001). As none of the above mentioned experiments applied to sunflower oil treatment of our study, we can only speculate that the plasma glucose lowering effect may depend on the fatty acid composition of the dietary fat.

The plasma triglyceride concentration in the sunflower oil group tended ($p < 0.1$) to be lower at the end of aqua training (sampling time T3 and T4; Table 8); while one hour after the training it was significantly lower compared to the other treatment groups. The triglyceride values measured in our trial were similar to those found for Thoroughbred racehorses (0.17–0.38 mmol/l; Li et al. 2012). Since the median of these values were more than twice as high as the control group of moderately exercised riding horses (0.284 vs. 0.128 mmol/l), the authors assumed that the racehorses exhibited an increased rate of lipid mobilisation. This explanation is supported by the result that triglyceride concentration increases during exercise as a function of the exercise intensity (Pösö & Hyyppa 1999). These results suggest that the muscles of racehorses adapt to high intensity exercise by gaining higher oxidative capacity and an increased capacity for fat utilisation as an energy source (Li et al. 2012). Based on that, we can conclude that the aqua treadmill training we applied can induce a similar triglyceride response, such as the Thoroughbreds' response to conventional training. Trained horses adapted to fat supplementation promote greater flexibility in the selection of substrate for exercise demand (Treiber et al. 2006, 2008). We believe that this adaptation was reflected in the lower plasma triglyceride level of the fat-supplemented group.

Table 8. Effect of dietary main energy source on the plasma triglyceride levels (mmol/L) of equine athletes during and after deep water aqua treadmill training.

Gait	Stand (preparation)	Walk (water)	Trot (water)	Walk (water)	Stand (under infrared)	Stand (in the box)	Stand (in the box)
Sampling time, min	0 (T0)	10 (T1)	40 (T2)	44 (T3)	60 (T4)	120 (T5)	180 (T6)
Control	0.398	0.418	0.435	0.438	0.358	0.343[a]	0.285
Sugar	0.375	0.408	0.390	0.410	0.295	0.323[a]	0.273
Oil	0.345	0.358	0.358	0.365	0.278	0.248[b]	0.255
Starch	0.313	0.353	0.340	0.365	0.300	0.318[a]	0.313
P_{model}	0.88	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{T0}		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$P_{treatment}$	0.88	0.20	0.13	0.07	0.09	0.03	0.21
RMSE	0.16	0.05	0.05	0.04	0.04	0.04	0.04

RMSE: root mean square error.
[a,b]Mean values within a column with similar superscript letters are not significantly different ($p > 0.05$).

Conclusions

Even the moderate difference in dietary energy supply of which could occur in practice can significantly modify some of the plasma blood parameters of equine athletes; however, the magnitude of these modifications is usually not considerable. A clear preference for any energy yielding substrate cannot be established; however some results indicate that higher starch content may help to reduce chronic muscle damage. It is clear that the training in water through its cooling effect results in markedly different lactate curves and values compared to conventional training. Therefore, the most often used lactate level is not a valid indicator of the workload strenuousness. Thus, other plasma parameters reflecting the workload like CK and AST should be examined as well.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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