THESES OF DOCTORAL (PhD) DISSERTATION

KAPOSVÁR UNIVERSITY

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FITNESS IMPROVEMENT OF SHOW JUMPERS BY HIGH INTENSITY AQUA TREADMILL AND FEEDING STRATEGY

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DOI: 10.17166/KE2016.009

KAPOSVÁR

2016

1. INTRODUCTION, AIMS

Equine athletes need training to achieve good performance in a similar way than Humans. The literature of equine athletes is relative abundant on data about Thoroughbreds, endurance and eventing horses. However only a few field tests can be found with show jumpers competing on lower level (Covalesky et al., 1992; Sloet van Oldruitenborgh-Oosterbaan et al., 2006; Soares et al., 2011), and more experienced horses competing in 130-150 cm high classes (Art et al., 1990[a,b]; Covalesky et al., 1992).

The idea of blood-based assessment of training effects, condition alterations and performance is certainly not new. Post-exercise blood lactate concentration is the most widely used indicator of horse fitness (Couroucé, 1999). Standard exercise tests provide the possibility of to run the horse under controlled conditions; however data collected from a treadmill test do not reflect the horse's response to a sport event. Horses are generally exercised on an open field or indoor, being exposed to numerous other factors such as the rider, other horses, weather, spectators, decorations, terrain, etc. (Serrano et al., 2001). Plasma lactate concentrations in Standardbred horses pulling a 10 kilopond draught load were lower on the treadmill than on the racetrack (Gottlieb-Vedi and Lindholm, 1997) and blood lactate in trotters were lower during exercise on a level treadmill than during exercise on a racetrack (Couroucé et al., 1999). In sport horses it has been also found that blood lactate concentrations were lower on the level treadmill compared with exercise over ground (Sloet van Oldruitenborgh-Oosterbaan and Barneveld, 1995). Therefore, testing the biochemical and physiological changes during field training or competition is important.

Hinchcliff et al. (2002) showed that the anaerobic capacity of horses could be increased by an appropriate conditioning program including regular and high intensity training. However, the regular high intensity conventional training may result in a large percentage of retirement from the training program due to injuries (Eto et al., 2004). The training in water was first applied in the rehabilitation of human athletes. The exercise of horses in water to improve fitness is not new, but recently there has been a development in the possible use of aqua treadmill for horses. Several studies had been performed with aqua treadmill (Lindner et al., 2010, 2012; Hevesi et al., 2009; Nankervis et al., 2008; Voss et al., 2002) to test its effect on metabolism using mainly heart rate and lactate as indicative variables. However, little information is available on the changes of other blood parameters.

Effect of training develops qualitative and quantitative changes in the blood, which means adaptation to the increased performance. Thus the relationship between blood biochemical parameters before and after exercise or competition can be important. However, few studies can be found in the literature that examine the correlation between blood parameters in endurance horses (Rose et al., 1986), Thoroughbred horse (Davie and Evans, 2000) and Italian Standardbred (Tateo et al., 2008).

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 et al., 1995[a,b]; Spangfors, 1998; O'Connor et al., 2001; Treiber et al., 2008). The daily rations of equine athletes should include a mixture of energy sources (starch, fat, fibre) in a balance (Pagan, 1998). Any extreme conditions in feeding (e.g. unbalanced energy supply) should be avoided. The cooling effect of water markedly alters the metabolic response of horses to aqua training was measured by various plasma biochemical parameters (Hevesi et al., 2009; Lindner et al., 2012). Thus, it can be hypothesised that the response of plasma biochemical parameters are altered by different dietary energy sources when deep water exercise is part of the training program.

Aims

The main aims of research project were the following:

- To study the effect of age and event on show jumpers plasma biochemical and enzyme activity parameters measured post competition.

- To study the effect of increasing aqua treadmill training intensity on the heart rate and several plasma biochemical parameter of show jumpers during aqua training and after competition.

- To examine the correlation between plasma biochemical parameters of show jumpers before and after deep water aqua training and jumping course completion.

- To determine the effect of different main dietary energy sources on several blood biochemical parameters on deep water aqua treadmill trained show jumpers using the energy source more diffused under field conditions.

2. MATERIAL AND METHODS

2.1. Experiment 1

2.1.1. Experimental animals

During the winter period (from October to February) the Indoor Show Jumping Championship is organized in Hungary. One location of the tournament is the Pannon Equestrian Academy at the Kaposvár University. Fifteen horses (n=15) were randomly selected in three age categories (five, six and seven years old, five animal/age group). We examined the same horse at the first (October 2009) and at the last (February 2010) event of the tournament.

2.1.2. Blood sampling

On the last day of the events, immediately after the first course two times 4 ml blood sample was taken from the jugular vein into the sampling tubes containing NaF-oxalate and Na-heparinate. The blood samples were stored on ice until we spinned them. The samples were spinned at 3000 rpm for 3 minutes. Plasma as pipetted to eppendorf tubes and stored at a temperature -18 ^{o}C until the analysis.

2.1.3. Laboratory analysis

From the blood plasma samples, activities of lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), levels of lactate, glucose, total cholesterol, triglyceride, total bilirubin and cortisol were determined in the laboratory of the Kaposi Mór Teaching Hospital (Kaposvár, Hungary) using Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring system.

2.1.4. Statistical analysis

The experimental data were evaluated by the SAS 9.1 (SAS Institute Inc., Cary, NC, USA) statistical software package with GLM procedure. Interaction of age and event effect was not significant in the case of any parameter; therefore it had been left out from the general model and results presented as pooled. In case of significant main effect, the differences between the group means were tested by Tukey-test. Discriminant analysis was used to test the hypothesis that treatments can be separated based on the blood parameters.

2.2. Experiment 2

2.2.1. Experimental animals

We examined four (three geldings, one stallion) normally trained show jumpers aged from 6 to 11 years at the Pannon Equestrian Academy, Kaposvár University.

Horses were housed individually in box (3m*3m). The daily feed allowance consisted of 12 kg meadow hay and 2.6 kg oat which provided 134.5 MJ DE and 1042 g crude protein. The horses had free access water and salt lick.

2.2.2. Training program

Horses were trained with high-intensity aqua treadmill in three periods during three days (Table 1). These horses did compete on the shows organized at the Equestrian Academy of Kaposvár as part of the Indoor Show Jumping Championship in Hungary. The horses finished one 110 cm high and 325 m / min class each day (Saturday and Sunday).

Immediately before the three test periods the experimental animals one times (Saturday and Sunday) competed the same class.

The training program was discussed with the riders and trainers in order to assure that aqua training may cause acceptable disturbance to the usual daily program. It was decided that horses should be aqua-trained between the morning and noon feeding.

Normal training was one hour training with rider, while jumping training was half an hour warming up and half an hour jumping training with rider.

Table 1: Training program of the 14 day experimental periods

	Days													
	M	T	W	TH	F	SA	S	M	T	W	TH	F	SA	S
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Normal training	X	X		X		X		X	X			X		
Jump. training			X		X					X				
Aqua training								X	X		X			
Compe-tition													X	X

M=Monday; T=Tuesday; W=Wednesday; TH=Thursday; F=Friday, SA=Saturday; S=Sunday

2.2.2.1. Aqua treadmill training

The protocol of the 44 minute long aqua training (performed three times a week) and blood sampling is reported in Table 2. During the aqua treadmill training the temperature of the water was 21 ^{o}C, while the level of the water was set to 15 cm above the shoulder joint (≈85% of the height at the withers). The temperature of the water was kept in reserve tanks on 21 ^{o}C constantly with a circular heating system to perform the same protocol. This program (Table 2) lasted 44 minutes: 10 minutes walking, 30 minutes trotting and 4 minutes walking. The maximum speed of the aqua treadmill increased from 9.0 to 11.0 and 13.0 km/h from training period to training period. Heart rate was monitored with Polar Equine RS800cx. After the aqua training horses were dried under infra-red lamps for about 16 minutes. The horses were then taken back to the stable.

2.2.3. Blood sampling

4 ml blood samples were taken during the aqua treadmill training program on Thursday at the time indicated in Table 2. These samples were taken from the jugular vein via catheters and placed into the sampling tubes containing NaF-oxalate or Na-heparinate. Additional blood sampling was carried out on both days of the event mornings and immediately after the first show jumping course (one times before the experimental periods - this data was the control - and three times the test periods) with venipuncture as suggested by Lindner et al. (1992). The blood samples were stored on ice until spinning. The samples were spinned at 3000 rpm for 3 minutes. Plasma were pipetted to an eppendorf tube and stored at a temperature -18 ^{o}C until the analysis.

2.2.4. Laboratory analysis

Same as in Experiment 1, see chapter 2.1.3.

Table 2: Protocol of high-intensity aqua treadmill training

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	9.0 /11.0 /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)	Relax in the box
6	120-180	-	180 (T6)	Relax in the box

* for the first, second and third experimental period, respectively

2.2.5. Statistical analysis

The experimental data were evaluated by the SAS 9.1 (SAS Institute Inc., Cary, NC, USA) statistical software package using CORR and the GLM procedure according to the following general model: $Y_{ijk} = \mu + I_i + T_j + (I*T)_{ij} + T0 + e_{ijk}$; where: μ = overall mean; I = intensity of aqua training (i=C,9,11,13 or 9,11,13); T = time of sampling (j=Saturday, Sunday or 10,40,44,60,120 min); I*T = interaction between aqua training intensity and time of sampling; T0 = covariate of the parameter's value measured at rest; e_{ijk} = residual error. The interaction was not significant ($P > 0.05$) in any case, therefore it was left out from the model and data presented as pooled. In case of significant treatment effect mean differences were tested by a Duncan multiple range test. Correlation coefficient was calculated with Pearson linear correlation.

2.3. Experiment 3

2.3.1. Experimental animals

We examined four (three geldings, one stallion) normally trained show jumpers aged from 7 to 12 years at the Pannon Equestrian Academy, Kaposvár University. The average body weight of the horses was 524±40 kg. Horses were housed individually in box (3m*3m). The horses had free access to water and salt lick.

2.3.2. 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 3) to provide different main energy sources but an identical amount of digestible energy (Table 4). The control group received the concentrate normally fed in the structure, while the three other concentrates provided an elevated levels of starch, total sugar and fat, respectively. The daily nutrient supply was sufficient or in excess to a horse with medium exercise intensity (NRC, 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.

Table 3: Feed allowance and composition of treatment groups (kg)

Feed component[a]	Treatments			
	Control	Starch	Total sugar	SF oil
Muesli[b]	0.25	0.20	-	-
Pelleted oats	1.25	2.05	0.50	0.50
Compound feed[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
SF oil = sunflower oil

Table 4: Daily nutrient intake of the treatment groups with hay and concentrate

Nutrient	Roughage[a]	Concentrate (according to treatments)			
	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 fiber, 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)
SF oil = sunflower oil

2.3.3. Training program

The horses (n=4) were trained according to the schedule presented in Table 5. Normal training was one hour training with rider, while jumping training was half an hour warming up and half an hour jumping training with rider. The protocol of the 44 minute long aqua training is reported in Table 4, the intensity of workload was 13 km/h in trotting.

Table 5: Training program of the 14 day experimental periods

	Days													
	F	SA	S	M	T	W	TH	F	SA	S	M	T	W	TH
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Normal training		X		X	X		X		X		X	X		
Jump. training	X					X		X					X	
Aqua training											X	X		X

F=Friday; SA=Saturday; S=Sunday; M=Monday; T=Tuesday; W=Wednesday; TH=Thursday;

2.3.4. Blood sampling

4 ml blood samples were taken during the aqua treadmill training program on Thursday at the time indicated in Table 2. These samples were taken from the jugular vein via catheters and placed into the sampling tubes containing NaF-oxalate or Na-heparinate. The blood samples were stored on ice until spinning. The samples were spinned at 3000 rpm for 3 minutes. Plasma were pipetted to an eppendorf tube and stored at a temperature -18 ^{o}C until the analysis.

2.3.5. 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 activities LDH, CK, AST, levels of lactate, glucose and triglyceride were determined using the Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring system.

2.3.6. Statistical analysis

The experimental data were evaluated by the SAS 9.1 (SAS Institute Inc., Cary, NC, USA) 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 Duncan multiple range test.

3. RESULTS

3.1. Effect of age and event on post exercise values of blood biochemical parameters in show jumping horses (experiment 1)

Five-year old horses had significantly lower lactate level than the six or seven years old horses. The average levels of the young horses (five years old) were within the usual range (ref. value 1.0-2.0 mmol/L) indicated for resting animals, while older horses (six, seven years old) developed a characteristic of post exercise level with the mean value of 3.5 mmol/L. The different results of the five years old horses can be answered by the difficulty of challenge they are facing with: the maximum height of the obstacles are 100 cm and the average minimum speed is 300 m/min, while for the six and seven years old horses it is 110 cm, 325 m/minute and 120 cm, 325 m/min, respectively. At the end of the tournament horses had lower glucose and higher cortisol level in the plasma right after show jumping class.

3.2. The effect of workload type and baseline covariate on the response of plasma biochemical parameters in show jumpers (experimtent 2)

Aqua training did result significant changes only in glucose, triglyceride and cortisol levels. In contrast show jumping resulted significantly higher level in all parameters measured except AST. We found positive correlation between same blood parameters before and after aqua training and competition in bilirubin, cholesterol, LDH, GOT, CK and cortisol. This information indicates that evaluating the effect of exercise on blood biochemical parameters can not be judged without the knowledge of baseline level. Our result clearly demonstrate that using baseline variables as covariate eliminates the significant individual effect.

3.3. Effect of deep water aqua treadmill training intensity on plasma biochemical parameters of show jumpers (experiment 2)

3.3.1. Aqua training

Heart rate elevated during the training according to the speed of treadmill from 4.5 km/h (walk) to 9/11/13 km/h (trot) and returned to normal level after the training. However, increasing the maximum speed of the aqua treadmill in phase of trotting (9km/h to 11/13 km/h) in successive experimental period had no influence on the heart rate average of horses subjected to the training. Plasma lactate level decreased with the increased maximum speed of the aquatrainer. Activities of AST, CK, LDH and value of cholesterol, cortisol and bilirubin decreased when the maximum speed of the treadmill was set to 11 km/h compared to the 9 km/h training. Interestingly, when the maximum speed was further increased to 13 km/h resulted in similar or sometimes higher values to those observed at the lowest training intensity. The levels of glucose and triglyceride increased when the maximum speed was changed from 9 to 11 km/h. However, when the speed was further increased to 13 km/h glucose level remained similar, while triglyceride values decreased to an even lower level measured at the lowest intensity aqua training. Plasma lactate and glucose levels decreased significantly during aqua training, and increased after the training. The triglyceride level elevated only at the end of the trotting phase and returned to resting level one hour after the training. The increased plasma cortisol level during aqua training shows that horses had stress situation. Other plasma biochemical parameters like

AST, CK, LDH, cholesterol and bilirubin had no response to aqua treadmill training.

3.3.2. Competition

Aqua training resulted in lower heart rate measured right after completing the show jumping course when horses were subject to medium intensity aqua training compared to the minimum intensity. Further increase in the training intensity had no effect on heart rate. Lactate level after competition were unchanged by aqua training, however the measured values were about two times higher compared to those experienced after one hour the aqua training. Activities of AST, CK, LDH and values of glucose and triglyceride were elevated when training of horses involved the lowest intensity of aqua training compared to the values measured as a result of conventional training (control). Increasing the aqua training intensity 9 to 11 km/h resulted in decreased activities of AST, CK and LDH, while levels of glucose and triglyceride remained unchanged after the competition. Aqua training had no influence on the levels of plasma cholesterol, cortisol and bilirubin measured after competition. Competing on consecutive days resulted in elevated AST and CK activities.

3.3.3. Correlation between plasma parameters during aqua training and after competition

Since aqua training had no or depressive effect on plasma biochemical parameters, only data of samples taken before aqua training (at rest - T0), after aqua training (standing under infrared lamps – T4) and one hour after aqua training (resting in the box – T5) was used for correlation analyses. Lactate level measured after competition had weak positive correlation only with plasma values obtained one hour after aqua training.

AST, CK and LDH activities had weak to moderate correlation, regardless to sampling time. Before the aqua treadmill training significant positive correlation was observed between AST - bilirubin, AST – triglyceride AST – CK and CK – bilirubin parameters. Furthermore, significant correlations were found between many other parameters like bilirubin – cortisol, cholesterol – LDH, AST – cortisol and CK – cortisol. We observed both before and after competition significantly positive and negative correlation between blood parameters. Cholesterol showed significantly negative correlation with: AST, CK, cortisol (before competition) and AST, CK, lactate and cortisol (after competition). Positive correlation was found between lactate – bilirubin, LDH – bilirubin, AST – lactate (before competition) and AST – lactate, CK – lactate, CK – AST, cortisol – lactate, cortisol – AST, cortisol – CK (after competition). Particularly strong correlation can be found between lactate and AST, CK and AST after competition.

The values of cholesterol and bilirubin had weak correlation to resting (T0) values. Cortisol level found after and one hour after aqua training did show weak correlation to values obtained after competition.

3.4. Effect of dietary energy source on the plasma parameters of equine athletes trained in a deep water aqua treadmill (experiment 3)

The different dietary energy sources resulted in similar plasma lactate level. The increased starch content of the feed resulted in significantly lower ($P<0.05$) creatine kinase activity at the end of the first walking section of aqua training. This result appeared later as a tendency ($P\leq0.1$). Horses fed sunflower oil as a main energy source had higher aspartate aminotransferase activity 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.

4. CONCLUSIONS AND RECOMMENDATIONS

Show jumping competitions on up to 100 cm height and a minimum of 300 m/min average speed do not involve substantial anaerobic energy metabolism. The frequent stress situation can elevate the cortisol response given by horses. Multivariate methods applied on several post exercise blood parameter can be useful to detect slight differences in fitness.

Different types of exercise results in different patterns of blood biochemical parameters, therefore results of different type of exercises cannot be compared directly. When evaluating the effect of exercise on blood biochemical parameters, the values measured before exercise should be used as covariate in order to get correct result.

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. Further studies needed to understand the metabolic processes altered due to the effect of water submersion.

The aqua training improves the fitness even of lower class show jumpers, through the improvement of oxidative energy regeneration. There is no plasma biochemical marker measured during or after aqua training which could predict fitness with sufficient accuracy. Therefore, monitoring of training progression still demands field tests.

Even the moderate difference in dietary energy supply of which could occur in practice can significantly modify some of the blood plasma 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.

5. NEW SCIENTIFIC RESULTS

1. Show jumping competitions on up to 100 cm height and a minimum of 300 m/min average speed do not involve substantial anaerobic energy supply in horses.

2. Due to the large individual variations in plasma biochemical parameters the resting values should be used as covariate when evaluating studies with equine athletes.

3. Plasma lactate alone does not reflect correctly the level of workload in case of high water level training, therefore measurement of several blood parameters is necessary (CK, AST).

4. Discriminant analysis can detect small differences in fitness.

5. Aqua training improves the fitness even of lower class show jumpers, through the improvement of oxidative energy supply.

6. PUBLICATIONS DERIVED FROM THE THESIS

6.1. Papers in scientific journals

Vincze, A., Cs. Szabó, Z. Bakos, V. Szabó, S. Veres, D. Ütő, Á. Hevesi
Effect of dietary energy source on the plasma parameters of equine athletes trained in a deep water aqua treadmill.
***ITALIAN JOURNAL OF ANIMAL SCIENCE*,** Published online: 23 Feb 2016.
http://www.tandfonline.com/doi/full/10.1080/1828051X.2015.1128688
IF 2015:0.841

Vincze, A., Cs .Szabó, V. Szabó, S. Veres, D. Ütő, Á. Hevesi
The effect of deep water aqua treadmill training on the plasma biochemical parameters of show jumpers.
AGRICULTURAE CONSPECTUS SCIENTIFICUS **78:**(3) *pp. 289-293.* (2013)

Vincze, A., Cs. Szabó, Á. Hevesi, S. Veres, D. Ütő, L. Babinszky
Effect of age and event on post exercise values of blood biochemical parameters in show jumping horses.
ACTA AGRARIA KAPOSVÁRIENSIS **14:**(2) *pp. 185-192.* (2010)

6.2. Full conference papers in proceedings

Vincze, A., Cs. Szabó, Á. Hevesi, S. Veres, D. Ütő

The effect of workload type and baseline covariate on the response of plasma biochemical parameters in show jumpers.

ACTA AGRICULTURAE SLOVENICA **100:**(Suppl. 3) *pp. 317-321*. (2012)

6.3. Submitted manuscripts

Vincze, A., Cs. Szabó, S. Veres, D. Ütő, Á. Hevesi

Fitness improvement of show jumper horses with deep water aqua treadmill. Submitted to Journal of Animal Physiology and Animal Nutrition. Submitted to Medicina Veterinara, IF 2015:0.560

7. OTHER PUBLICATION

7.1. Full conference papers in proceedings

Pastva, A., Cs. Szabó, A. Vincze, M. Baban, B. Antunovic, P. Mijic

The effect of training method on the condition of horses.

In: Sonja Marić, Zdenko Lončarić

Zbornik radova [Proceedings]: 48. Hrvatski i 8. Međunarodni simpozij agronoma [48th Croatian and 8th International Symposium on Agiculture]. 925 p.

Conference place and date: Dubrovnik, Croatia, 18.02.2012.-22.02.2012. Osijek: Poljoprivredni Fakultet Sveučilišta Josipa Jurja Strossmayera, 2013. pp. 785-789.

(ISBN:978-953-7871-08-6)