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

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ABBREVIATIONS

ADP	Adenosine diphosphate
ALT	Alanine transaminase
AST	Aspartate Aminotransferase
ATP	Adenosine triphosphate
CK	Creatine kinase
CORR	Procedure to analyse correlation in SAS (SAS Institute Inc., Cary, NC, USA) statistical software
DE	Digestible energy
FFA	Free fatty acid
GLM	General linear model
HDL	High-density lipoproteins
IDL	Intermediate-density lipoproteins
LDH	Lactate dehydrogenase
LDL	Low-density lipoproteins
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NEFA	Non-esterified fatty acids
NRC	National Research Council
PCr	Phosphocreatine
RBC	Red blood cell
$V_{LA1.5}$, V_{LA2}, V_{LA4}	Velocity at which plasma lactate concentration reached 1.5, 2 and 4 mmol/L
VFA	Volatile fatty acid
VLDL	Very-low-density lipoproteins

1. INTRODUCTION

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 levels (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, 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 and Jackson, 1995; Pagan et al., 1995; 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.

2. REVIEW OF THE LITERATURE

2.1. Muscle fiber types and energy systems

Muscle fibers are usually grouped as Type I, Type IIA, and Type IIX (Rivero et al., 1999). Interconversions between Type IIA and IIX (IIAX) are well recognized in the literature and it exist in equine locomotory muscles in significant numbers (Dingboom et al., 1999; Linanne et al., 1999). Type I (slow twitch, slow-oxidative) fibers are highly oxidative, meaning they use aerobic metabolism to produce energy-generating ATP (slow ATP hydrolyzes) and are well equipped to use fat as a substrate. These fibers have a small cross-sectional area, a high number of capillaries and high oxidative capacity but their glycolytic capacity and glycogen content are relatively low. Type I fibers are highly efficient and economical in producing slow repetitive movements and sustaining isometric force that do not require great force generation. Type II fibers (fast twitch, fast-oxidative glycolytic) are subdivided into Type IIA (both high and low oxidative) and Type IIX (low oxidative, fast glycolytic) fibers. The type IIA fibers have a considerable number of both capillaries and mitochondria and rely on glycolytic and oxidative metabolism. These fibers capable of utilizing both aerobic and anaerobic metabolism to produce energy for work and it used to maintain high speed or jumping. Type IIX fibers are used to give the horse speed and it have a maximal velocity of shortening that is three times higher than that of IIA fibers (Rome et al., 1990) and it typically extract energy from anaerobic glycolysis. Type II fibers, particularly the type IIX fibers, are more suited to rapid contraction and high force generation and thus must be used during speed or strength work (Yamano et al., 2006). Type IIX fibers are highly glycolytic and, thus, prefer carbohydrate as an energy source over

fat. II AX fibers are intermediate in their properties (Quiroz-Rothe and Rivero, 2001). Type I fibers have greater lipid storage than Type IIA fibers, while there is negligible triglyceride in Type IIX fibers. Type IIX fibers have higher glycogen stores than Type IIA fibers, while Type I fibers glycogen content are relatively low.

At low intensity work (walk) the muscle contraction is slow and it uses only small amounts of ATP. This exercise operates primarily the Type I muscle fibers and generates energy through aerobic pathway using fats. When the speed (intensity) is increased (trot, canter) the Type I muscle fibers are not able to contract with appropriate speed, therefore, it will be involved in the type IIA muscle fibers. These fibers can also fuel from aerobic and anaerobic phatways using fat and glycogen also. The importance of this that from glycogen can be produced ATP two times faster than from lipids (Taylor et al., 1974). As soon as the intensity of exercise is further increased (fast gallop) the type IIX fibers also come into muscle work and it gains energy from anaerobic glycolysis. The anaerobic glycolysis is the fastest way to generate energy, however, as a consequence accelerates the accumulation of lactic acid in the muscles and decrease of muscles pH (Pagan, 1998).

There are existing differences in the ratio of Type I to Type II muscle fibers among breeds of horses, more specifically, among types of performance (Snow and Valberg, 1994). Arabians or Andalusians have a lower proportion of Type II muscle fibers when compared to Quarter Horses and Thoroughbreds (Snow and Guy, 1980; López-Rivero et al., 1990).

2.1.1. The main energy sources

Adenosine triphosphate (ATP) is the main source of readily available chemical energy in cells. At the beginning of any exercise energy is provided by ATP stores and the ATP-creatine phosphate pathway. However, these energy supplies deplete rapidly as a result of work. Therefore, it can provide the energy requirement only for 1-2 minutes (Ellis and Hill, 2005). Cells generate ATP on one hand from oxidative phosphorylation - catabolism of carbohydrates, fats, and very small amounts of proteins - using oxygen (aerobic energy production) on the other hand from glycolysis - breakdown of glucose and glycogen produce energy (lactic acid is produced, anaerobic energy production). Lactate threshold is defined as the point when lactic acid starts to accumulate and the plasma lactate concentration reaches about 4 mmol/L (V_{LA4}) in human (Heck et al., 1985) and equine (Evans et al., 1995), which is known as the anaerobic threshold. However, Castejón et al., (1994) 2 mmol/L (V_{LA2}) and Lindner (2010) 1,5 mmol/L ($V_{LA1.5}$) were found to best represent the aerobic – anaerobic lactate threshold of horses. Lactate threshold is useful indicators of aerobic capacity (represent the maximal work intensity at which ATP is produced aerobically) and are frequently used in the evaluation of fitness and state of training. This threshold varies and depends on several factors such as the muscle's fiber-type composition, level of fitness and the diet. Fat- rich diet promotes oxidative energy production via free fatty acids (FFAs) (Orme et al., 1997) thereby increasing the oxidative capacity of muscle (Geelen et al., 2001[a]; Dunnett et l., 2002) and sparing glycogen (Geelen et al., 2001[b]). Carbohydrates and fats are the predominant sources of ATP under normal condition. The balance between carbohydrate and fat utilization may be influenced by the physiological status of the horse, feeding state, type of diet and physical

conditioning. Substantial source of energy is the muscle triglycerides store (~ 2000g), glycogen (~ 3500g), triglycerides stored in fat deposits (~ 40000g) and glycogen stored in the liver (~ 150g) (Pagan, 1998).

Glucose is the primary source of energy for somatic cells used for ATP production. Muscle and liver glycogenolysis starts to occur soon after the start of aerobic exercise. The transport of carbohydrates in the plasma glucose content is mainly achieved by means of a permanent and continuous source of energy for all tissues (Gaál, 1999). Cells may obtain glucose from the circulation or from intracellular stores of glycogen (liver, muscle). Glucose in the circulation may originate from hepatic glycogenolysis, from hepatic gluconeogenesis (carbohydrates and their metabolites - lactate, pyruvate, oxaloacetate ect., glucogenic amino acid, odd chain fatty acids) or from food consumed and digested by the horse. Glucose can be catabolized for ATP production in two ways; one way is the most efficient method of ATP production (1 glucose generates 36 ATP) it requires oxygen, the other way can be done in the absence of oxygen, which is less efficient (1 glucose generates 2 ATP), where pyruvic acid is converted into lactic acid. When oxygen becomes available, lactic acid is converted back to pyruvic acid. The major energy sources for oxidation for muscle cells are plasma glucose and free fatty acids (Vervuert, 2011). During prolonged submaximal exercise, blood glucose may still account for up to 25% of the total energy output (Valberg, 1996).

The long-chain fatty acids utilized by cells may originate from recently consumed food, but most of the long-chain fatty acids that are oxidized for energy probably originate from either intracellular stores or adipose tissue. When the body requires fatty acids as an energy source, the hormone glucagon signals the breakdown of the triglycerides by

hormone-sensitive lipase to release free fatty acids (Elsersawi, 2013). The triglycerides in adipose tissue are broken down to long-chain non-esterified fatty acids and glycerol that are released into the blood. The triglycerides of the chylomicrons in the blood are broken down (lipoprotein lipase enzyme) in adipose tissue and muscle tissue to long-chain fatty acids and glycerol too. The glycerol component of triglycerides can be converted into glucose via gluconeogenesis, while the fatty acids are catabolized by Beta-oxidation in the mitochondria to be entered into the Citric Acid Cycle as two-carbon fragments and it can generate 17 molecules of ATP. Lipolysis requires more oxygen and occurs much more slowly than equal carbohydrate metabolism.

Non-esterified fatty acids (NEFA) are molecules released from triglycerides by the action of the enzyme lipase and are transported in the blood bound to albumin by hydrophobic forces in plasma. It is contribute only a small proportion of the body's fat, however they provide a large part of the body's energy. During low- to moderate-intensity exercise, there is a progressive increase in lipid oxidation with increasing exercise duration (Pagan et al., 1987; Rose et al., 1991). NEFA are important for the physical performance during the aerobic exercise of short intensity and long duration (Piccione et al., 2009). Triglyceride are released from the liver as very-low-density lipoproteins (Pösö et al., 1989) and may have been synthesized in response to increased delivery of NEFA to the liver. Hyyppä et al. (1997) demonstrated by decreases in NEFA concentrations during intense exercise bouts. Lipoprotein lipase located on the outer side of the endothelial membranes in muscle capillaries will release fatty acids from circulating VLDL (very-low-density lipoproteins) for oxidation in muscles.

Short-chain (volatile) fatty acids can also be used for energy production. Most volatile fatty acids (VFA) originate from the large intestinal fermentation of carbohydrates. VFA production in the cecum may be sufficient to meet up to 30 percent of a horse's energy needs at maintenance (Glinsky et al., 1976). Experimental results demonstrated that horses consuming a diet composed primarily of hay will meet more than 80 percent of their energy needs from VFAs (Vermorel et al., 1997). VFAs may be available as energy sources to cells or they may be metabolized to long-chain fatty acids or glucose. Acetate is the predominant VFA produced in the large intestine, but significant amounts of propionate are also generated. Pethick et al. (1993) demonstrated that acetate oxidation might contribute about 30 percent of the energy utilized by the hind limb at rest. Other experiment has suggested that up to 50 – 60 percent of circulating glucose in forage-fed ponies originates from absorbed propionate (Simmons and Ford, 1991).

The body prefers not to use protein for energy because it is specifically needed to build and repair all kinds of tissues. If the body do not have enough carbohydrates or fats to cover the energy requirements, amino acids from dietary protein are converted into energy. In order to use amino acids as energy from the glucogenic amino acid process of removing that amino group is (deamination - taking away the amino group, $-NH_2-$), and the resulting ketoacids getting involved in carbohydrate metabolism and it synthesized glucose.

2.2. Performance tests

Sport physiology is the youngest branch of physiological sciences. Its subject is to study the physiological changes caused by physical exercise. Physiological studies systematize the normal functions of the organism

and sport physiology deals with its enhanced operation. Exercise physiology can be classified as one of the applied physiological sciences, because it uses the methods of classical physiology for studying functions caused by physical exercise and sport (Kenney et al., 2015).

Performance-physiological examinations - besides measuring physical performance - makes it possible to define adaptational processes (metabolic, cardio-vascular, skeletal) and based on them, it is possible to suggest an efficient training programme both for amateur and professional sportsmen.

During physical exercise acute changes occur in the different organ systems (cardio-vascular, muscular system, etc.). The extent of these changes depends on various factors: the form, duration and intensity (strain, frequency) of workload and on the individual differences (Krumrych, 2006). The organs and their functions adapt to regular physical activity. Depending on the duration and regularity of physical strain, we can differentiate between acute effect and chronic adaptational processes.

While planning and carrying out performance tests, we need to keep in mind that we will have to be able to answer the following simple questions of the rider, the trainer or the owner (Hinchcliff et al., 2008):

- What fitness level does my horse have actually?
- Has the fitness level of the horse changed as a result of the specific training work?
- Is the horse fit enough for its next race?
- What changes should be made so that my horse would reach a better fitness level?

- Can weaker performance be a result of an inadequate training programme or can it be caused by some health problem?

The biggest challenge of equine exercise tests is the standardisation of exercise bouts. In the course of field training, due to the individual differences in the riders' skills, canter and trot length and speed, and other environmental factors the very fully identical test cannot be ensured. Therefore the development of treadmills suitable for animal tests opened new possibilities in equine studies. It does not mean that we do not need field tests. We can mention advantages and disadvantages of both testing types. A basic requirement of the tests is to be well-planned and repeatable.

2.2.1. Treadmill tests

To be able to compare several horses' performance, or the performance of one horse on different days, it is necessary to standardise the conditions of performance, which requires the use of a high-speed treadmill. Modern high speed treadmills are able to reach 17 m/s speed, however, for reliable results, maximum effort tests are often done at a lower speed, and at an incline of more than 10% (Hinchcliff et al., 2008).

The advantages of treadmill testing are obvious: the tests can be conducted under controlled conditions, one can control the duration of the test, or the speed of the horse's movement; in addition, environmental factors such as temperature and humidity can be kept at a constant level. As several other factors (like the effects of the environment, that of the rider or that of the trainer), can be eliminated, treadmill testing is an important means of sport physiological research.

Establishing in standardized conditions can help to predict performance. Measurements can be carried out easily, it is possible to check heart rate and breath frequency continuously during movement (Persson, 1983), or to measure the activity of different muscle groups (Van Wessum et al., 1999), or to examine the breathing gas exchange (Persson et al., 1980; Rose and Evans, 1987). A further advantage is that taking blood samples, is possible during training, without the need to stop the movement of the horse (Baragli et al., 2001). We can repeat the training programmes under the same circumstances as many times as we need to, and during testing we can change one particular factor, while the other factors remain constant. If necessary, we can enhance strain by putting the required amount of weight on the horse, or we can do the testing with the assistance of an experienced rider (Sloet van Oldruitenborgh-Oosterbaan and Barneveld, 1995). It is also possible to increase strain further by the increase of the treadmill's angular offset, which can create the effect of the horse going upgrade.

The disadvantage of treadmill training is that the horse is not in its natural surroundings during the test, so measurements do not evaluate the effects of normal, everyday training. Moreover, we cannot disregard the fact that both the biomechanical variables of locomotion (Buchner et al., 1994) and the strain are different in the case of treadmill tests and field tests (Sloet van Oldruitenborgh-Oosterbaan, 1999). Exactly that is the reason why the results of field and treadmill tests are not comparable (Sloet van Oldruitenborgh-Oosterbaan and Barneveld, 1995; Couroucé et al., 1999). A further drawback of this method is the high investment cost, and the maintenance cost of the equipment. Before doing the clinical test, the horse must be acclimatized to the treadmill, for which we have to spend enough time, as the time of acclimatization to these artificial surroundings

is different among horses (King et al., 1995; Scheffer and Sloet van Oldruitenborgh-Oosterbaan, 1996).

A special type of the treadmill is the deep water treadmill, which in fact is a conveyor belt operating under a water column at variable height and temperature values; it can help training horses in their regeneration or rehabilitation (Sloet van Oldruitenborgh-Oosterbaan and Barneveld, 1995; Gottlieb-Vedi and Lindholm, 1997). Earlier, the equipment was used to post-operational or post-traumatic rehabilitation, during which cure rate increased and the rehabilitation period decreased. The hydrodynamic force reduces the strain on the joints, and makes it possible to develop the muscles. Nowadays it is not used only for rehabilitation but also for physiological examinations all over the world (Nankervis et al., 2008; Lindner et al., 2010). The results of deep water treadmill tests cannot be compared directly either to treadmill or to field tests (Knudsen and Jørgensen, 2000).

2.2.2. Field tests (track tests)

During field tests the horse and the rider do their training in familiar surroundings, on the ground, so the tests and their results are closer to the everyday work, and are more similar to the circumstances the horse probably meets during a competition. The rider influences his/her horse during these tests, just like in everyday work or at a race. Another advantage is that the horse does not need to be acclimatized before doing the test.

However, during these tests controlling the speed can be problematic even in the case of an experienced rider (Davie and Evans, 2000); moreover, significant influencing factors are the outside temperature, the wind, the humidity, the quality of the soil, the weight of the rider and his/her riding

technique; all of these influence the test results (Hargreaves et al., 1999). A further difficulty lies in sampling, as the horse has to stop for taking each blood sampling, and thus the strain (the speed) cannot be increased as evenly as in the case of the treadmill. The stops, however they are short, can affect the blood concentration of metabolites (Hinchcliff et al., 2008). In contrast, several tests prove that field tests are reliable and repeatable and natural (Kobyashi et al., 1999; Couroucé, 1999).
A special form of field tests is when the examinations are done during a competition (Art et al., 1990[a,b]). The racing situation usually causes bigger stress for the horse than the everyday training, so the test results differ from an average field test, as well (Couroucé, 1999).

Performance tests can help to predict the performance of the horse, and they can also point out health problems, which lie behind poor performance. During the planning of each test (type, duration, etc.) we try to find the answers to a particular question, where results must be given so that they help the rider, the trainer or the owner to reach better fitness and better performance.

2.3. Assessment of the physical fitness by blood plasma biochemical parameters

In most of the studies the heart rate and blood plasma lactate measurements are the bases of an exercise test evaluation for training horses (Covalesky et al., 1992; Couroucé et al., 2000). Other researchers documented that in the physiological control of physical fitness the enzymes of blood plasma are also important, especially lactate dehydrogenase (LDH), transaminase enzymes (AST, ALT) and creatine kinase (CK). These parameters change as the effect of training

(Brancaccio et al., 2008) or injury. Studies with horses indicated that the responses of blood biochemical parameters to different exercises vary (Davie and Evans, 2000; Soares et al., 2011). Several studies have demonstrated the extent of changes depends on several factors: type of exercise, intensity of work (strength, duration and frequency) and individual variation (Krumrych, 2006). In this section systematized the changes of the blood biochemical parameters as the result of exercise in horses.

2.3.1. Lactate

Lactate has two optical isomers, and in animals the L-lactate is constantly produced from pyruvate via lactate dehydrogenase (LDH). This is a process of fermentation during normal metabolism and exercise too. The concentration of blood lactate is usually 1–2 mmol/L at rest (Hinchcliff et al., 2008) and a normal resting plasma lactate concentration (Table 1) is approximately 1.5 mmol/L in horses (Nappert and Johnson, 2001), but can be up to 20 mmol/L as the effect of intensive training (Evans and Golland, 1996). Accumulation of lactic acid occurs when there is a high demand for energy (intensive training) but the supply of oxygen to the cells is limited, such as what happens during anaerobic exercise. In this case the lactate is produced faster than the ability of the tissues to remove it; therefore the blood lactate concentration starts to rise. This is a beneficial process since the regeneration of NAD+ provides (pyruvate is reduced to lactate while NADH is oxidized to NAD+) that energy production is maintained and training can continue. The increased lactate level can be removed for oxidation to pyruvate by well-oxygenated muscle cells, heart cells, and brain cells and then it can be directly used to fuel the Krebs cycle. The other ways the lactate conversion to glucose via

gluconeogenesis in the liver and release back into (Cori cycle) circulation (McArdle et al., 2010).

Several studies have dealt with lactate in blood tests. Research of Art et al. (1990[a,b]) shows that the post competition blood plasma lactate level was between 6-9 mmol/L for horses competing on 130-150 cm obstacles with above 350 m/min speed (Table 2). In horses, the dynamic and periodic contractions necessary to clear the obstacles during a jumping competition could also induce a large decrease in the blood supply of the working muscles. This fact together with the high metabolic requirement of muscular contraction during the jump could explain the increase in lactate and the high heart rate recorded in horses competing on higher classes (Art et al., 1990[b]). Surprisingly, it can be noticed among show jumpers that the speed and the duration of this kind of competition is provided not only through oxidative processes, but also to a great extent through anaerobic metabolism, with lactate formation (Art et al., 1990[b]). Based on more than five competitions (Art et al., 1990[b]) no difference was found in post-competition blood plasma lactate level. The exercise over 800 m at speeds 780-960 m/min resulted in 4-19 mmol/L blood lactate level immediately after the exercise (Davie and Evans, 2000). Muñoz et al. (2008) determined the maximum blood plasma lactate concentration after the exercise, which was 13.7 mmol/L in draft horses, 12.8 mmol/L in racing horses and 2.9 mmol/L in endurance horses. Snow and MacKenzie (1977) demonstrated blood plasma lactate concentration of more than 7mmol/L in less than 3 min as a result of high intensity training programme, which consisted of gallop or trot at high speed.

Several studies describe the effects of training on the relationship between blood lactate and velocity in horses (Anderson, 1975[a]; Wilson et al., 1983; Bayly et al., 1987; Lindner et al., 1992). Furthermore, field tests have been

used to investigate the relationships between speed, heart rate and blood lactate concentrations in the Thoroughbred horse (Anderson, 1975[a]; Bayly et al., 1987; Evans et al., 1993; Harkins et al., 1993; Wittke et al., 1994; Guhl et al., 1996). For the training of horses the heart rate and the lactate level responses to speed are important indicators for determining the level of training, health status and physical fitness (Art et al., 1994; Couroucé, 1999; Couroucé et al., 2000; Hebenbrock et al., 2005). Traditionally, heart rate and lactate have been studied, reported and correlated as a function of speed (Persson, 1997). Other researchers observed highest correlation with blood lactate concentration after 2 and 5 min post-exercise on treadmill (Evans et al., 1993). The onset of blood lactate accumulation has been defined as a threshold reached when the horses are running at 350 to 400 m/min, the levels of blood lactate and heart rate are expected to rise above 4 mmol/L and 150 to 160 beats/min, respectively (Persson, 1997). Davie and Evans (2000) measured relatively high (r = 0.75) correlation between velocity and blood lactate concentration.

Several studies have demonstrated that physiological responses to treadmill training can not be compared to responses of field training. Sloet van Oldruitenborgh-Oosterbaan and Barneveld (1995) found in Warmblood horses that heart rate and blood plasma lactate concentrations were lower on the treadmill test at speeds of 6.5–9.4 m/s compared with exercise over ground. However, when the treadmill incline increased to 1–2% or the speed of treadmill increased by 10% it induced the same heart rates as in the field. In Standardbred horses pulling a 10 kilopond draught load the plasma lactate concentrations were lower on the treadmill than on the racetrack (Gottlieb-Vedi and Lindholm, 1997), blood lactate in trotters was lower during treadmill training than during exercise on a racetrack (Couroucé et al., 1999). Hevesi et al. (2009)

demonstrated that plasma lactate level decreased during deep water aqua treadmill training and elevated during the resting period. Lindner et al. (2012) using 20 min exercise period, 19.8 km/h maximum speed and water height at 80% of the withers height did not observe markedly greater blood lactate level (1.5-2.0 mmol/L) after the exercise. However, higher lactate values were found when the height of water was only at the height of 50 and 65% of the height of withers compared to the level at 80% (Lindner et al., 2010). However, Lindner et al. (2012) measured 1.23 to 1.4 mmol/L blood lactate level during high level aqua training. Studies have shown that the temperature of the water plays an important role in the development of cardiac rhythm (Nankervis et al., 2008). The thermoneutral zone for horses in air is ranging from 5 to 25 °C (Morgan, 1998), but it in water has not been established yet (Lindner et al., 2012), this value in humans ranges from 33 to 35 °C (Choukroun and Varene 1990).

Several studies have shown that lactate accumulation increased during repeated sprints, when the horses were fed with 10% corn oil instead of a diet without added fat (Ferrante et al., 1993; Kronfeld et al., 1994; Taylor et al., 1995). Contrary to that Julen et al. (1995) observed no effect of fat feeding on blood plasma lactate concentrations in horses. Similar results were found by Harkins et al. (1992) in a 1600 m race and by Scott et al. (1992) in repeated sprints. Sloet van Oldruitenborgh-Oosterbaan et al. (2002) demonstrated lower blood plasma lactate accumulation when the horses were fed with a high-fat (11.8% in dry matter) instead of a low-fat (1.5% fat) diet when the horses were trained with a standardized sub-maximal exercise test.

Table 1: Reference values of horses

Lactate	1.5 mmol/L (Nappert and Johnson, 2001)	1–2 mmol/L (Hinchcliff et al., 2008)		
LDH	74-206 U/L (Lumsden et al., 1980)	162-412 U/L (Kaneko et al., 2008)		
CK	11-130 U/L (Lumsden et al., 1980)	2.4 - 23.4 U/L (Kaneko et al., 2008)	210 U/L (Pritchard et al. 2009)	90-270 U/L (Adamu et al., 2013)
AST	86-552 U/L (Lumsden et al., 1980)	226-366 U/L (Kaneko et al., 2008)		
Glucose	4.16-6.39 mmol/L (Kaneko et al., 2008)			
Cortisol	36-81 nmol/L (Kaneko et al., 2008)			
Cholesterol	1.94-3.89 mmol/L (Kaneko et al., 2008).			
Triglyceride	0.1-0.5 mmol/L (Kaneko et al., 2008)			
Bilirubin	7.1-34.2 µmol/L (Kaneko et al., 2008).			

2.3.2. Lactate dehydrogenase (LDH)

LDH present mainly in animals (skeletal and heart muscle, liver, red blood cells, etc.) and even detected in plants (O' Carra and Mulcahy, 1996). The tissue destruction associated with the extent and course of diseases such as heart attack, hepatitis, tumor, muscle damage or haemolysis can be estimated by measuring LDH activity. This enzyme is involved in glucose metabolism of all cells. Thus, this is the key enzyme

in the bloodstream of tissues having high glucose metabolism such as liver, heart and skeletal muscle, red blood cells, nerve tissue and tumor tissue. LDH catalyses the interconversion of lactate and pyruvate and with parallel interconversion of NADH and NAD+. It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased, thus lactate to pyruvate is preferred. In this respect the elevated activity of LDH is the result of anaerobic energy supply. The reference values (Table 1) varies between wide ranges such as 162-412 U/l (Kaneko et al., 2008) or 74-206 U/l (Lumsden et al., 1980). The LDH values may be higher in case of haemolysis (disintegration of red blood cells), heart attack, hepatitis, muscle damage, pancreatitis or tumor.

The LDH activity increased significantly due to show jumping exercise compared to pre-event values (Art et al, 1990[b]). Guy and Snow (1977) described that a marked increase in lactate dehydrogenase activity is the effect of high intensity exercise. For example, immediately after training or competition its value can increase up to twice and after 12 hours it can be three times higher compared to baseline value (Gaál, 1999). However, Fregin and Thomas (1983) have shown that the conversion of lactic acid to pyruvate does not keep pace with the growing lactic acid production. It is assumed, however, that the elimination of lactic acid and its breakdown rate was faster with higher LDH activity. This is supported by Kovács (2006) who also established that the increased LDH activity coupled with lower lactate level due to faster elimination results in more quick recovery. Many factors can influence the plasma activity of LDH such as age, sex, exercise and training programs. Muñoz et al. (2002)

demonstrated that elevated plasma muscle enzyme is more prevalent in mares than in male horses, suggesting a hormonal predisposition. Other researchers demonstrated that most probably genetic (Gaffey and Cunningham, 1988) and/or other factors like nutrition are responsible for the activity of LDH, while exercise has little influence on it. The LDH activity increased as the effect of training plus some supplementary feed, but it cannot be excluded that the increased LDH activity in the development of the English thoroughbred horses is also partly a genetically fixed property (Gaffey and Cunningham, 1988). Plasma activity of LDH and other enzymes (i.e., AST, CK) is increased (Table 2) typically in horses during jumping or endurance exercise (Balogh et al., 2001; Krywanek et al., 1996) and similar findings have been reported after varying short distance exercise (Cornelius and Kaneko, 1963; Anderson, 1975[b]) and endurance rides (Rose et al., 1977).

2.3.3. Creatine kinase (CK)

Creatine kinase, also known as creatine phosphokinase (CPK) is an enzyme responsible for the energy production of cells. CK catalyses the conversion of creatine and utilizes ATP to create phosphocreatine (PCr) and ADP. This enzyme reaction is reversible and thus CK regenerates ATP from ADP, using PCr. The source of CK activity that can be measured in the blood plasma of healthy individuals is almost entirely from skeletal muscle. The high activity occurs with either skeletal or heart diseases (myocardial infarction, myocardial inflammation), muscle injury, burns, etc. Usually this enzyme is used to detect damages of the heart or skeletal muscle (Gaál, 1999). A high CK activity, or the increase over subsequent measurements generally indicates that there has been some damage to the heart or other muscles. It can also indicate that the muscles

have undergone heavy load. Various reference ranges (Table 1) can be found in the literature for CK: 2.4 - 23.4 U/L (Kaneko et al., 2008); 11-130 U/L (Lumsden et al., 1980) and 90-270 U/L (Adamu et al., 2013). Pritchard et al. (2009) determined a 210 U/l reference value for Lahore working horses (Pakistan).

The increase of creatine kinase activity in healthy horses indicates the intensity of the workload, and can easily double effects of training compared to the activity measured in animals resting (Art et al., 1990[a]). The increase CK and LDH activities in the serum, immediately after exercise, could be mainly because of a selective increase of muscle membrane permeability (Anderson, 1975[b]). Balogh et al. (2001) observed significantly increased activity of CK immediately post-exercise compared with pre-exercise samples in Pentathlon Horses. Prolonged endurance exercise can result in very high CK activity (1000 – 30000 U/L) without signs of macroscopic muscle damage (Kerr, 1983; Adamu et al., 2013). CK activity are increased typically in horses during jumping or endurance exercise (Balogh et al., 2001; Krywanek et al., 1996) and similar findings have been reported after varying short distance exercise (Cornelius and Kaneko, 1963; Anderson, 1975[b]) and endurance rides (Rose et al., 1977). Interestingly, significantly lower CK activity were measured (Table 2) in show jumpers performing higher class competitions (Art et al., 1990[a,b]). Other experimental results are also indicating that excessive training does not result in an increase of CK activity (Harris et al., 1997; Hamlin et al., 2002). In another study show jumping test failed to further increase elevated CK activity in the serum (Soares et al., 2011). Pritchard et al. (2009) measured high activity of CK and it is probably the result of low-level but chronic muscle injuries (caused by the everyday work itself performed by those horses), and not a

reversible result of a single exhaustive exercise bout. This assumption was based on the fact that the reference population was working daily for short periods. These observations indicate that even horses prone to regular, but relatively short intensive exercises could have a chronic muscle damage, which results in somewhat elevated CK activity (about 200-300 U/L). It has been documented that overtraining has not resulted in elevated CK activity (Harris et al., 1997; Hamlin et al., 2002). Rumley et al. (1985) demonstrated that total CK activity after 30 minutes or 30 hours post-race do not correlate with finishing time of endurance horses.

2.3.4. Aspartate aminotransferase (AST)

AST can be found also in the liver, kidneys, brain, red blood cells, heart and skeletal muscles, and elevated activity can indicate muscle damage due to muscle strain from exercise. The increased activity can be caused by liver damage as well (Gaál, 1999). AST catalyzes the reversible transfer of the amino group from aspartate or glutamate to the corresponding ketoacid, as such, is an important enzyme in both amino acid degradation and biosynthesis. In amino acid degradation, following the conversion of α-ketoglutarate to glutamate, in the reverse reaction, aspartate may be synthesized from oxaloacetate, which is a key intermediate in the citric acid cycle. A wide range of reference values (Table 1) can be found in the literature for AST: 226-366 U/L (Kaneko et al., 2008) and 86-552 U/L (Lumsden et al., 1980).

Art et al. (1990[a,b]) observed significantly lower AST activity in higher class show jumping horses (Table 2). However, in English thoroughbred horses with good racing results continuously elevated (around 300 U/L) AST activity were found (Harris et al., 1990). As AST is also mainly released from muscle, the simultaneously elevated activities with CK

indicates a strenuous exercise. Freestone et al. (1989) measured in nine Thoroughbred horses increased AST and CK activities immediately after and 4 hours after 400 m and 1000 m test. Siciliano et al. (1995) observed that even submaximal exercise can elevate serum AST (and CK) and that conditioning can reduce these responses. Andrews et al. (1995) measured significantly higher activity of AST (and CK activity) after exercise in endurance competition compared to the eventing competition. Tateo et al. (2008) demonstrated that there was no correlation between the method of conditioning and the activity of AST (and CK, LDH) in the field test in Italian Standardbred horses.

Valentine et al. (1998) fed 19 horses with a high-fat (9-11% in dry matter) diet for 3-6 months and measured that all horses had abnormal glycogen accumulation and serum CK and aspartate transaminase (AST) activities four hours after exercise. Post-exercise CK and AST activities after feeding the high-fat diet were significantly lower than the values before training.

Table 2 : AST, CK LDH activities and lactate concentrations after exercise

Type of horse	Type of training	Lactate (mmol/L)	AST (U/L)	CK (U/L)	LDH (U/L)	R
Standardbred trotters	2100 m	B. 21.6 ± 0.9 P. 24.5 ±0.7	-	-	-	1.
Thoroughbred	sand surface 800m 15.6 m/s	B. 9.2	-	-	-	2.
	grass surface 800m 15.6 m/s	B. 14.5	-	-	-	
Thoroughbred races	1100-3800 m	B. 29.6 ± 4.7 P. 33 ± 1.9	-	-	-	3.
Polo	cross country event	P. 9.2 ± 1.2	-	-	-	4.
Standardbred	Treadmill 6.4 m/s	P. 27.7	-	-	-	5.
Standardbred trotters	Treadmill 9.8 m/s 4 % incline	P. 13	-	-		6.
Spanish Purebred Foals	Treadmill 6 m/s 6 % incline	P. 9.45 ± 3.24	253 ± 113.21	144.55 ±58.86	607.07 ±197.72	7.
Spanish Purebred Adult	Treadmill 6 m/s 6 % incline	P. 9.15 ± 4.33	236.28 ±55.20	129.10 ± 47.14	588.53 ±238.39	
German Warmblood riding horse	Treadmill water 50% of the withers	P. 1.9	-	-	-	8.
Show Jumping	150 cm high obstacles	P. 9	120	80	350	9.
Show Jumping	110 cm high obstacles	P. 3.9 ±0.7	362 ±65.4	195 ± 13	505 ± 48	10.
Show jumping horses	90 cm high obstacles	P. 3.86	-	-	-	11.
	120 cm high obstacles	P. 2.83	-	-	-	
	140 cm high obstacles	P. 4.48	-	-	-	

P.: plasma lactate; B.: blood lactate; AST: aspartate aminotransferase; CK: creatine kinase, LDH: lactate dehydrogenase; values are means ± standard error of the mean; - not measured

R=references; 1: Pösö et al., 1995; 2: Davie and Evans, 2000; 3: Harris and Snow, 1988; 4: Geiser et al., 1994; 5: Gauvreau et al., 1996; 6: Couroucé et al., 2000; 7: Rubio et al., 2008; 8: Lindner et al., 2012; 9: Art. et al., 1990[a]; 10: Soares et al., 2011; 11: Covalesky et al., 1992

2.3.5. Glucose

It is the primary source of energy for body cells (Van Soest, 1994). It is transported from the intestines or liver to body cells via the bloodstream, and is absorbed by body cells with the intervention of the hormone insulin normally produced by the body. For muscle work it is crucial to use sugar and the continuous reload of glycogen stores. Glycogen - glucose polysaccharide molecules - can be found in all cells, but glycogen can only be stored in the liver and muscles. Reference range (Table 1) is 4.16-6.39 mmol/L (Kaneko et al., 2008).

Long workload (more than 3 hours) decreases the plasma glucose level, but a shorter training can decrease and increase as well depending on the intensity of workload and dietary energy source (Pösö and Hyyppä, 1999; Snow and MacKenzie, 1977). Art et. al. (1990[a]) measured similar values at rest (5.43 mmol/L) and after higher show jumping course (5.05 mmol/L). The same phenomenon has been observed in some horses after canter (Anderson, 1975[a]). Blood glucose has been reported to increase following racing and three-day event competition, and to fall with endurance exercise (Lindholm et al., 1974; Rose et al., 1980; Snow et al., 1983). Contrary to that, Andrews et al. (1995) measured significantly higher level of glucose after exercise in Endurance competition compared to the eventing competition. Experimental results have demonstrated that when carbohydrates are substituted with fat (oil) on isocaloric bases, the blood glucose and insulin levels are decreasing (Pagan et al., 1995). Lower glucose level was observed after dry treadmill training of Thoroughbreds when 15% of the daily energy intake was provided as oil (Crandell et al., 1999). When oil substitutes soluble carbohydrates (starch, sugar) in the feed, the adaptation processes reduce the glucose substrate (carbohydrate to lipid shift in the metabolism) dependence of the work

(Treiber et al., 2008). The quality (fatty acid composition) of the dietary fat source modifies the glucose metabolism. Fish oil supplementation resulted in lower glucose level compared to the corn oil fed group (O'Connor and Lawrence, 2004).

2.3.6. Triglyceride

From a biochemical viewpoint, fats belong to a broad group of compounds known as lipids that can be glycerol or nonglycerol based (such as waxes, steroids, alkalines etc.). Glycerol-based lipids can be categorized simple and complex. Simple lipids contain only glycerol and fatty acids (such as monoglyceride, triglycerides), while complex lipids include glycerol, fatty acids plus another nutrient group (such as phospholipid, glycolipid). The main biological functions of lipids include energy storage, signalling, and acting as structural components of cell membranes (Fahy et al., 2009; Subramaniam et al., 2011). Triglycerides consist of three fatty acid molecules linked (ester bonds) to a glycerol backbone. Fatty acids may be grouped on the basis of the number of carbon atoms they contain and the number of double bonds (saturated or unsaturated). Very long chain fatty acids contain more than 22 carbon atoms, long-chain fatty acids contain 13 to 21 carbon atoms, medium-chain fatty acids contain 6 to 12 carbons, and short-chain or volatile fatty acids (VFAs), produced in the intestinal tract by bacterial fermentation, contain only 2 to 5 carbons. The fat, liver and gut endothelial cells can synthesize and the former store triglycerides. The reference range (Table 1) is 0.1-0.5 mmol/L (Kaneko et al., 2008).

Lipoproteins are complex aggregates of lipids and proteins (transported within the protein outer shell) which enable fats to be carried in the blood stream. The role of plasma lipoprotein particles is to transport

triglycerides, cholesterol, and phospholipids in the blood between all the tissues of the body. Lipoproteins are synthesized in the small intestine (travel into the blood stream via the lymphatic system) and the liver (released into the blood). Plasma lipoprotein particles can be classified based on their relative densities of the aggregates on ultracentrifugation HDL (high-density lipoproteins), LDL (low-density lipoproteins), IDL (intermediate-density lipoproteins), VLDL (very-low-density lipoproteins) and ULDL (commonly called chylomicron) lipoproteins. In adipose tissue, hydrolysis of fats free fatty acids are released into the blood stream where they bind to albumin.

Pösö et al. (1983) documented that the triglyceride concentration of blood depends on the genotype, because the ability of liver to metabolize triglyceride varies between horse breeds. Carreón et al. (2013) documented that the age of horses does not affect the level of triglyceride (Mayer et al., 1984), and the castration does not increase the triglyceride level, while in fleshy horses they measured significantly higher triglyceride level compared to moderate and thin horses. Similarly, Ju et al. (1993) did not find differences between different sexes of horses in triglyceride level. In contrast, Nazifi et al. (2005) documented increased triglyceride level with age. Nevertheless, Gupta et al. (2002) measured higher triglyceride level in 6 month old foals compared to 3 year old horses.

During high intensity training greater utilization of triglycerides can be observed (Li et al., 2012), and the triglyceride concentration decreases as the effect of constant training (Kedzierski and Podolak, 2002; Muñoz et al., 2002; Kedzierski et al., 2009). The triglyceride concentration increases in blood plasma after exercise as a function of the exercise intensity (Pösö and Hyyppä, 1990). Several studies demonstrated

(Kedzierski and Podolak, 2002; Muñoz et al., 2002; Kedzierski et al., 2009) that the high intensity training can cause higher triglyceride values which concentration decreases after several months of the training. But the level of triglycerides decreased after aqua training of Thoroughbred race horses (Li et al., 2012). These results suggest that race horse muscles are adapting to high intensity exercise by gaining higher oxidative capacity and an increased capacity for fat utilization as energy source (Li et al., 2012). Asadi et al. (2011) measured in Arab horses bred in Iran 1.92 mmol/L triglyceride level. Nevertheless, the physical activity (frequency and intensity of training) had no effect on serum triglycerides (0.31 mmol/L) of horses in Colima, Mexico (Carreón et al., 2013). Nevertheless, Viana et al. (2007) documented that the elevated intensity of exercise increased the level of triglycerides. Furthermore, Pérez et al. (1997) measured 0.28 mmol/L level of triglycerides in Chilean sport horses.

Trained horses adapted to fat supplementation promote greater flexibility in the selection of substrate for exercise demand (Treiber et al., 2008; Treiber et al., 2006). Sloet van Oldruitenborgh-Oosterbaan et al. (2002) demonstrated significantly lower concentration of triglyceride in pre-exercise when the horses fed a high-fat (11.8% in dry matter) instead of a low-fat (1.5% fat) diet, when the horses were trained a standardised sub-maximal exercise test. Horses consuming fish oil had lower blood triglyceride concentration compared to those fed corn oil (O'Connor and Lawrence, 2004). This result indicates that not only the level of dietary fat, but its fatty acid profile also influences the plasma triglyceride level.

2.3.7. Cholesterol

Cholesterol is a waxy steroid (nonglycerol based lipid) of fat that is synthesized in the liver or intestines. It is important within cells, and it is a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D (Hanukoglu, 1992). Although cholesterol is important and necessary for mammals, high level of cholesterol in the blood can clog arteries and are potentially linked to diseases such as those associated with the cardiovascular system (heart disease). Cholesterol is only slightly soluble in water; it can dissolve and travel in the water-based bloodstream at exceedingly small concentrations. Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins. Reference range (Table 1) is 1.94-3.89 mmol/L (Kaneko et al., 2008) in the blood.

Carreón et al. (2013) documented that the age of horses does not affect the level of cholesterol (Mayer et al., 1984). In contrast, Nazifi et al. (2005) documented that the level of cholesterol increases with age. Nevertheless Gupta et al. (2002) measured higher cholesterol level in 6-month old horses compared to 3 year old horses. Cell proliferation requires cholesterol, therefore higher cholesterol level can be expected in younger animals.

Cholesterol level in blood plasma increases as a result of training but not due to conditioning (Hambleton et al., 1980). Nevertheless, Lopez et al. (1974) demonstrated that cholesterol level decreases as the effect of exercise. While Asadi et al. (2011) measured 1.92 mmol/L cholesterol level in Arab horses bred in Iran. Sloet van Oldruitenborgh-Oosterbaan et al. (2002) demonstrated significantly higher concentration of cholesterol (3.00 ± 0.47 and 2.11 ± 0.49 mmol/L, respectively), HDL cholesterol (1.80 ± 0.18 and 1.35 ± 0.27 mmol/L, respectively) at rest in horses fed a

high-fat (11.8% in dry matter) instead of a low-fat (1.5% fat) diet when the horses were trained with a standardised sub-maximal exercise test (Mayer et al. 1984).

2.3.8. Cortisol

It is a corticosteroid hormone or glucocorticoid produced by *zona fasciculata* of the adrenal cortex, which is a part of the adrenal gland. Its primary function is to increase blood sugar and stores of sugar in the liver as glycogen (Hoehn and Marieb, 2010), and also suppresses the immune system. The cortisol level in the blood varies during the day. The reference range (Table 1) is 36-81 nmol/L (Kaneko et al., 2008). Abnormally elevated cortisol level can be observed in clinical depression, psychological stress, and such physiological stressors as hypoglycemia, illness, pain, trauma, surgery, fear, fever, physical exertion or extremes of temperature. Increased serum concentration may be due to muscle damage or to injury of organs containing smooth muscle (Stockham and Scott, 2002).

Cortisol is increased in the horse during a wide variety of exercise activities (Hyyppä, 2001; Horohov et al., 1999). Release of cortisol allows an individual to tolerate and adapt to challenges to homeostasis that occur in everyday life (Willmore and Costill, 1994; Thornton, 1985). Exercise has been associated with increase in plasma cortisol concentrations in many species including man (Farrell et al., 1983) and horse (Linden et al., 1991) and responses both exercise intensity and duration. The elevated cortisol level during aqua training indicates that this type of training does pose a stress situation on the horses, even if they had past experience. However, Marc et al. (2000) measured cortisol peak after the dry treadmill training. Plasma adrenaline, noradrenaline, beta-

Endorphin and cortisol concentrations were increased by training in cool dry conditions (cortisol: 90 ng/ml) and were further increased by the same exercise in hot humid (cortisol: 130 ng/ml) conditions (Williams et al., 2002). Art and Lekeux (1995) reported increases in plasma cortisol after treadmill exercise in hot and humid environment compared to temperate conditions. The plasma cortisol concentration better reflects duration of the workload rather than the work intensity (Saastamoinen and Martin-Rosset, 2008). The cortisol level has been shown to be involved in response to exercise and thermoregulation, although information regarding endocrine responses to the combined effects of exercise, heat stress and acclimation in man (Moseley, 1994; Mora-Rodriguez et al., 1996) and horse (Art and Lekeux, 1995) are limited. Maximum plasma concentration was observed about 30 min after the end of a high intensity exercise (Marc et al., 2000). Other researchers observed the maximum plasma concentration 5-30 min after the end of a short high intensity exercise (Jimenez et al., 1998; Nagata et al., 1999; Marc et al., 2000). It was demonstrated that discrimination based on cortisol net increase (due to exercise) between endurance and dressage plus jumping trained horses are possible. Covalesky et al. (1992) observed that more experienced horses have lower cortisol concentrations than less experienced ones after riding the course. Based on Coenen's (2005) studies different metabolic stresses – like jumping – can double the level of plasma cortisol.

2.3.9. Bilirubin

It is the breakdown product of haemoglobin. Based on studies of Ralston and Larson (1989) and Gaál (1999) increased bilirubin level occurs if the horse is worked beyond what it is capable of, creating muscle damage and red cell damage too, or if there is a problem with the bile ducts or the

liver. The reference range (Table 1) is 7.1-34.2 μmol/L (Kaneko et al., 2008).

2.4. Conclusions from the literature

As a result of exercise several blood biochemical parameters are changes, however, most studies deal with lactate only. Lactate level can vary in a wide range, which depends on the intensity, duration and type of exercise. Therefore, the characterization of lactate response to aqua training and the measurement of other biochemical parameters are necessary. Since plasma parameters response to treadmill training does not reflect in field tests, the evaluation of deep water aqua treadmill training in competition environment is necessary. Dietary energy sources can affect the metabolic response to exercise, thus testing feeds varying in main energy source is necessary to better understand the metabolic changes occurring during and as a result of aqua training.

3. OBJECTIVES OF THE DISSERTATION

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.

4. MATERIAL AND METHODS

4.1. Experiment 1

4.1.1. 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.

4.1.2. 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.

4.1.3. 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. Tests measuring principle - Bilirubin:

photometric, Dichlorophenyl - Diazonium (DPD) method; Lactate: enzymatic assay, in a colorimetric (570 nm) method; Glucose: coupled enzyme assay, in a fluorometric (587 nm) method; Triglycerides: photometric, GPO-PAP method; Cholesterol: Photometry, enzymatic CHOD-PAP method; LDH: photometric, according to the DGKC recommendations optimized standard method.; AST: Photometry, according to IFCC recommendations optimized kinetic UV test.; CK: photometric, IFCC reference method, UV test. Cortisol: electrochemiluminescence immunoassay (ECLIA), biotinylated polyclonal anti-cortisol antibodies.

4.1.4. 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.

4.2. Experiment 2

4.2.1. 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.

4.2.2. Training program

Horses were trained with high-intensity aqua treadmill in three periods during three days (Table 3). 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.

Table 3: 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

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.

4.2.2.1. Aqua treadmill

4.2.2.1.1. Technical data

- Useful outside length: 4.5 m
- Outside length: 4.7 m
- Useful insider width: 0.92 m
- Outside width (supporters, service pathway): 3.4 m
- Inside height over the belt: 2.0 m
- Maximum water level above the belt: 1.6 m
- Maximum outside height: 2.58 m
- Empty weight: approx. 3.5 t

4.2.2.1.2. Construction

The bottom tank was made of rigid, carbon-steel profiles, lined with wnr 1.4301 quality stainless steel plates. The water flow into through the 5 stubs (60 mm diameter each), which are evenly spread under the belt. Two openings are found for cleaning.

Side walls with doors were built with stainless steel lining (Figure 1). The doors are closed with 6 screw shafts or quick shafts each to ensure proper sealing. The walking surface is a 4mm thick plate. The frame is connected to the bottom tank on 16 points.

At the bottom 1000 mm wide, 9 mm thick, endless rubber belt can be found. The speed of the belt can be smoothly adjustable between 0 km/h

and 15.5 km/h. Speed can be set with a 0.1 km/h accuracy. This is provided by a driving gear powered by an electric motor (output: 11 kW, rpm output: 166 1/min).

The water circulates in a closed system (Figure 2). First the water is filtered mechanically in two steps after the training (net filtering, Triton 60 sand filter), after this the mechanically filtered water is sterilized by UV-light, its pH is corrected (7.2-7.6) and is oxygenized in order to obtain the proper quality so it can be used again. Water is circulated by two pumps. One with a higher output ($30m^3$/hour) fills the tank of the treadmill quickly (80 cm raise of the water level in 5.5 minutes) and a smaller pump ($15m^3$/hour) circulates the water in the reserve tank to ensure filtration.

The heat regulation is supplied by a 35 kw counter-current heat exchanger, which receives hot water from a water heater (gas, electric or alternative energy). Temperature can be adjusted on the control panel of the heating unit.

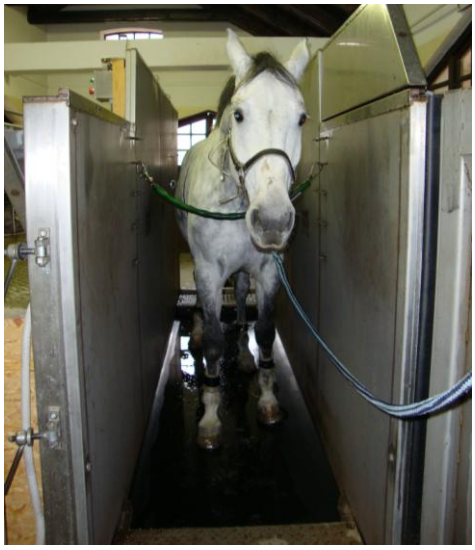

Figure 1: Aqua treadmill with a horse participating in the experiment

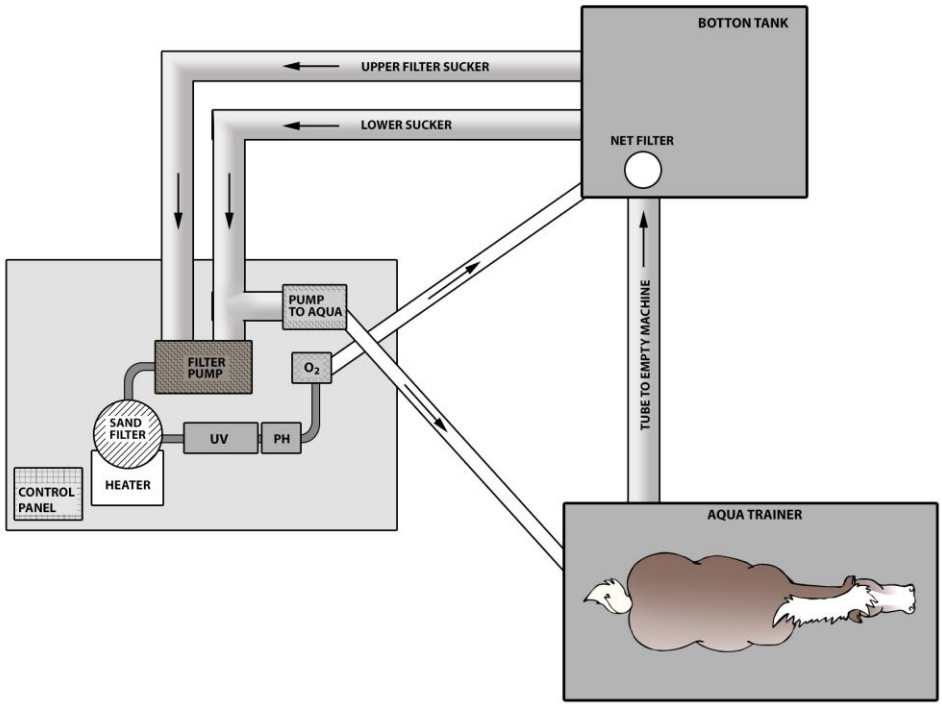

Figure 2: The construction of aqua treadmill

4.2.2.1.3. Training program of deep water aqua treadmill

The protocol of the 44 minutes long aqua training (performed three times a week) 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 15 cm above the shoulder joint (≈85% of the height at the withers, Figure 3). 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 (Table 4) 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 (Figure 4). After the aqua training horses

were dried under infra-red lamps for about 16 minutes (Figure 5). The horses were then taken back to the stable.

Figure 3: The level of the water was set to ≈85% of the height at the withers during the deep water aqua treadmill

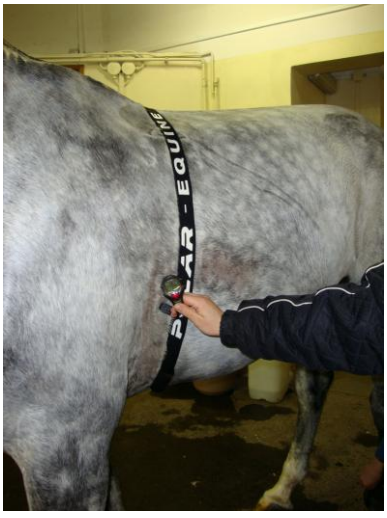

Figure 4: Heart rate monitoring with Polar Equine RS800cx

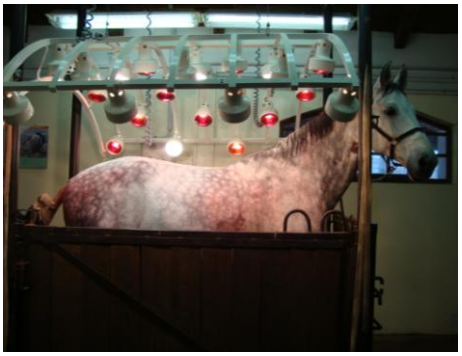

Figure 5: After the training the horses were dried under infra-red lamp

Table 4: 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

4.2.3. Blood sampling

4 ml blood samples were taken during the aqua treadmill training program (Figure 6) on Thursday at the time indicated in Table 4. 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.

Figure 6: Blood samples were taken from the jugular vein via catheters during the deep water exercise

4.2.4. Laboratory analysis

Same as in Experiment 1, see chapter 4.1.3.

4.2.5. 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=Sat,Sun or 10,40,44,60,120); 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.

4.3. Experiment 3

4.3.1. 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, it was calculated according to the equation of Wagner and Tyler, (2011). Horses were housed individually in box (3m*3m). The horses had free access to water and salt lick.

4.3.2. 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 5) to provide different main energy sources but an identical amount of digestible energy (Table 6). 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 5: 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 portions 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 6: 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

4.3.3. Training program

The horses (n=4) were trained according to the schedule presented in Table 7. 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 minutes long aqua training is reported in Table 4, the intensity of workload was 13 km/h in trotting.

Table 7: 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=Thursdays;

4.3.4. Blood sampling

4 ml blood samples were taken during the aqua treadmill training program on Thursday at the time indicated in Table 4. 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.

4.3.5. 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 of lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), levels of lactate, glucose and triglyceride were determined using the Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring system.

4.3.6. 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.

5. RESULT AND DISCUSSION

5.1. 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 compared to the 6 and 7 years old horses (Table 8). The average levels of the young horses were within the usual range (reference range 1.0-2.0 mmol/L) indicated for resting animals, while older horses developed a characteristic of post exercise level with the mean value of 3.5 mmol/L. Art et al. (1990[a,b]) measured post competition lactate level between 6-9 mmol/L for horses competing on 130-150 cm obstacles with above 350 m/min speed. Davie and Evans (2000) demonstrated that exercise over 800 m at speeds 780-960 m/min results in blood lactate concentrations in the range of 4-19 mmol/L measured right after the exercise. The maximum lactate concentration determined by Muñoz et al. (2008) after exercise in draft horses were 13.7 mmol/L, in racing horses were 12.8 mmol/L and in the endurance horses it was 2.9 mmol/L. The high intensity training programme consisting of gallop or trot at high speed resulted in a blood lactate concentration of more than 7 mmol/L in less than 3 min (Guy and Snow, 1977). These results indicate that lower class show jumping (up to 120 cm height) is not a strenuous exercise to horses. Lactate threshold is defined as the point when lactic acid starts to accumulate and the plasma lactate concentration reaches about 4 mmol/L. (Evans et al., 1995), which is known as the anaerobic threshold. Lactate threshold is a useful indicator of aerobic capacity and are frequently used in the evaluation of fitness and state of training. The low energy providing capacity over time of the aerobic pathways is the answer why lactic acid pathway is used. The results of older show jumping horses support that theory. The warm up

period usually lasts for about 30 minutes and involves light work and then riding the course takes 60-90 seconds as intensive work. The different results of the 5 years old horses can be answered by the difficulty of challenge they are facing with: the maximum height of the obstacles is 100 cm and the average minimum speed is 300 m/min, while for the 6 and 7 years old horses it is 110 cm, 325 m/min and 120 cm, 325 m/min, respectively. The oxygen consumption during exercise is known to be close correlation with the energy expenditure during work. Eaton et al. (1995) and Pagan and Hintz (1986) estimated the energy expenditure based on oxygen uptake. They estimated similar values at the speed of 300 m/min but markedly different at 400 m/min. These results suggest that up to 100 cm class competitions the aerobic energy providing pathway can meet the energy requirements of the horse.

At high concentrations of lactate, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased, thus lactate to pyruvate is preferred. In this respect the elevated activity of LDH (reference value 74-206 U/L, Lumsden et al., 1980) is the result of anaerobic energy supply, however the differences in lactate level of age groups did not reflected in mean LDH activity. Interestingly, Art et al. (1990[a,b]) found lower LDH activity in spite of higher post exercise lactate values, however horses were competing on higher classes.

The elevated activity of creatine kinase reflects the intensity of actual workload and it can be easily double as seen in endurance horses and in our trial (Table 8). Again, Art et al. (1990[a,b]) measured much lower post exercise CK activity in horses competing in higher classes. It has been documented, that overtraining is not resulted in elevated CK activity (Harris et al., 1997; Hamlin et al., 2002). Nevertheless Pritchard et al. (2009) determined a 210 U/l reference value for Lahore working horses

(Pakistan). They stipulated that the high activity of CK in working horses may indicate a low-grade, chronic muscle damage rather than a short-term and reversible effect of overwork. This assumption was based on the fact that the reference population was working for short periods daily. Elevated AST activity and total bilirubin level can indicate muscle damage due to muscle strain from use, training. Our results fall in the wide reference ranges set for both light and working horses (Lumsden et al., 1980; Pritchard et al., 2009), but Art et al. (1990[a,b]) measured noteworthy lower AST activity in high class show jumpers. Nevertheless, Harris et al. (1990) found persistently elevated AST (above 300 U/l) in thoroughbred racehorses with good race results. Art et al. (1990[b]) concluded that a single blood sampling could lead to a misinterpretation of post exercise biochemical values due to high interindividual variations. This regards particularly to blood lactate, LDH and CK. This phenomenon implies that the ability to compare different research results based on these biochemical parameters is limited.

All horses total cholesterol level were between the reference value, however it tended to be higher at the end of the season. It has been shown that cholesterol level in blood plasma increases as a result of exercise (maintenance of cellular membrane integrity) but not due to conditioning (Hambleton et al., 1980).

Plasma adrenaline, noradrenaline, beta-Endorphin and cortisol concentrations were increased by exercise in cool dry conditions (cortisol: 90 ng/ml) and were further increased by the same exercise in hot humid (cortisol: 130 ng/ml) conditions (Williams et al., 2002). The degree of increase in plasma cortisol concentration appears to better reflect duration of workload rather than work intensity. Maximum plasma concentration was observed about 30 min after the end of a high intensity exercise

(Marc et al., 2000). It has been also revealed that discrimination based on cortisol net increase (due to exercise) between endurance and dressage plus jumping trained horses are possible. Covalesky et al. (1992) demonstrated that more experienced horses have lower cortisol concentrations than less experienced ones after riding the course. In our study we were not able to detect this difference. Most probably the reason is that even young (5 year old) horses were already accustomed to the show environment.

Time of measurement (effect of event) had significant effect only on the blood glucose and cortisol levels. One of the primarily function of cortisol is to increase blood sugar level for instance in case of physical exertion. That can be the reason why we found significantly higher value, while blood sugar level was lower. Cortisol plays important role in adaptation to stress. As summarized by Coenen (2005) different metabolic stresses – like jumping – can double the level of plasma cortisol. The significantly higher level at the last competition of the tournament can be the result of higher metabolic stress. Most horses did compete every second week during the winter season which together with the frequent transportation can cause higher reaction to these stress. Blood lactate concentration tended to be higher at the last event. Art et al. (1990[b]) found no difference in post competition lactate level over five competitions.

Table 8: Blood parameters of different aged show jumpers at the beginning and at the end of winter competition period (mean values)

Blood parameter	Age (year)			Event		RMSE	P values (age)	P values (event)
	5 (n=10)	6 (n=10)	7 (n=10)	First (n=15)	Last (n=15)			
Lactate (mmol/L)	1.5[a]	3.5[b]	3.5[b]	2.3	3.4	1.6	0.010	0.059
LDH (U/L)	539.7	589.4	572.5	577.9	556.5	115.0	0.623	0.614
CK (U/L)	241.5	243.8	211.8	231.5	233.3	39.5	0.151	0.902
AST (U/L)	281.5	317.7	275.5	283.3	299.8	58.7	0.239	0.449
Glucose (mmol/L)	3.9	4.6	4.4	4.7[a]	3.8[b]	0.9	0.171	0.007
Chol (mmol/L)	2.3	2.3	2.4	2.3	2.5	0.3	0.607	0.098
Trig (mmol/L	0.36	0.30	0.42	0.37	0.35	0.12	0.100	0.651
Bilirubin (µmol/L)	27.7	29.8	32.2	28.2	31.4	7.1	0.376	0.220
Cortisol (nmol/L)	197.7	199.6	170.8	167.0[a]	211.7[b]	42.2	0.252	0.007

[a.b] Means within a row lacking a common superscript differ significantly $P<0.05$, RMSE: route mean square error
Chol=cholesterol, Trig=triglyceride,

Since few significant differences were found with analyses of variance, we hypothesized that treatment groups may be still possible to be distinguished based on all the traits measured. Therefore, discriminant analysis was applied on the data set. We are aware that in this study we had too few observations compared to the number of traits to make firm conclusions on the result of this statistical analysis. Moreover statistically proven outliers were not excluded, because those values were physiologically sound. Therefore, even significant results are interpreted as a tendency.

Three data were misclassified from the event groups of data (Table 9), and Pillai' Trace statistics show weak discriminant function. None of the data were misclassified from age groups (Table 10) and statistics indicates a strong discriminating function. It is a general view that only post exercise plasma lactate level and heart rate are good indicators of fitness, and other biochemical and enzymatic activity analyses are of limited usefulness (Art et al., 1990[a]; Art et al., 1994; Couroucé, 1999). However, our results indicate that the difference in fitness and metabolic status based on various biochemical parameters of different aged show jumpers could be possible in spite of few significant differences.

Table 9: Classification results of sampling time groups

from event group	into event group				Total		Pillai' Trace	
	first event		last event					
	count	%	count	%	count	%	value	P value
first event	14	93.33	1	6.67	15	100		
last event	2	13.33	13	86.67	15	100		
Total	16	53.33	14	46.67	30	100	0.55	0.030

Table 10: Classifications results of age groups

from age group (year)	into age group (year)						Total		Pillai' Trace	
	5		6		7					
	c	%	c	%	c	%	c	%	value	P value
5	10	100	0	0	0	0	10	100		
6	0	0	10	100	0	0	10	100		
7	0	0	0	0	10	100	10	100		
Total	10	33.33	10	33.33	10	33.33	30	100	0.93	0.040

c=count

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

Aqua training did result significant changes only in glucose, triglyceride and cortisol levels (Table 11). In contrast show jumping resulted significantly higher level in all parameters measured except AST. These differences are in agreement with other scientific results confirm that various exercises result in different responses of blood parameters (Gottlieb-Vedi and Lindholm, 1997; Couroucé et al., 1999; Sloet van Oldruitenborgh-Oosterbaan and Barneveld, 1995). Hevesi et al. (2009) and Voss et al. (2002) demonstrated similar effect compared to dry treadmill exercise. The lack of clear metabolic response after aqua training most probably is a result of the cooling effect of the water, which limits the speed of biochemical processes. The appropriate temperature, the continous more intensive flexor-extensor exercise, the massage effect of water and increased capillary activity must be important factors to explain the lower lactate-level during aquatrainig (Hevesi et al., 2009).

Valette et al. (1993) estimated the anaerobic treshold about 2.25 mmol/l. In this respect the competition resulted anaerobic muscle work, despite the short intensive work. In our previous study (Vincze et al., 2010) we measured higher post competition level of lactate (3.5 mmmol/l) for conventionally trained show jumpers competing on 110 and 120 cm class. Art et al. (1990[a]) found about 9 mmmol/l post competition lactate level for horses competing in 150 cm classes. These results suggests that there must be a close correlation between the effort required to pass the obstacle (height of the obstacles) and the lactate response. Release of cortisol allows an individual to tolerate and adapt to challenges to homeostasis that occur in every life (Willmore and Costill, 1994; Thornton, 1985). The level of cortisol is increased in the horse during a wide variety of exercise activity (Horohov et al, 1999; Hyyppä 2001; Snow and Rose, 1981), and the release appears to be affected by both intensity and duration of exercise (Thornton, 1985; Snow and MacKenzie, 1977). In our study both types of exercise significantly increased the level of cortisol. Interestingly, our data shows that the aqua training was a more stressful exercise, based on both pre- and post-exercise values. However, if we calculate the response given it is similar.

Table 11: The effect of training method and individual variance on some plasma biochemical parameters in show jumpers (n=12)

Parameter	Sampling				Horse				Effect		
	BA	AA	BC	AC	A	B	C	D	**S**	**H**	**S*H**
Bilirubin (μmol/L)	15.0^{ab}	15.9^{ab}	14.0^{b}	17.0^{a}	15.2^{ab}	16.5^{a}	17.4^{a}	13.0^{b}	***	***	NS
Glucose (mmol/L)	4.8^{ab}	4.0^{c}	5.0^{a}	4.5^{b}	4.6	4.7	4.6	4.7	***	NS	NS
Lactate (mmol/L)	0.63^{b}	0.40^{b}	0.87^{b}	2.41^{a}	1.0^{b}	1.0^{b}	2.0^{a}	1.0^{b}	***	***	***
Triglyceride (mmol/L)	0.36^{b}	0.44^{a}	0.33^{b}	0.45^{a}	0.34^{b}	0.39^{ab}	0.41^{a}	0.42^{a}	***	***	NS
Cholesterol (mmol/L)	2.1^{b}	2.0^{b}	2.1^{b}	2.2^{a}	2.3^{a}	2.3^{a}	1.8^{c}	2.1^{b}	***	***	NS
LDH (U/L)	668^{ab}	611^{b}	623^{b}	735^{a}	751^{a}	618^{b}	615^{b}	680^{ab}	**	*	NS
AST (U/L)	308^{b}	294^{b}	324^{ab}	356^{a}	294^{b}	286^{b}	415^{a}	313^{b}	***	***	NS
CK (U/L)	232^{b}	205^{b}	237^{b}	280^{a}	186^{c}	225^{b}	316^{a}	253^{b}	***	***	NS
Cortisol (nmol/L)	155^{b}	216^{a}	120^{c}	176^{b}	137^{b}	152^{b}	199^{a}	154^{b}	***	***	***

BA – before aqua training, AA – after aqua training, BC – before competition, AC – after competition;
H – horse, S – sampling; NS – not significant; * - P<0.05; ** - P<0.01; *** - P<0.001
^{a,b,c} Means in a row of an effect lacking a common superscript differ (P < 0.05)

Similarly to the observation of Hevesi et al. (2009) we also found significant individual (horse) effect in the case of most parameters measured. The differences between horses often was higher than the response given to the competition as an excercise. This high individual variation can reduce the number of significant differences in studies where the number of experimental units is rather limited. Grigoriev et al. (1995) demonstrated in humans that there is a correlation between blood biochemical parameters before and after physical challenge. We found positive correlation between bilirubin, triglyceride, cholesterol, LDH, AST, CK and cortisol before and after treadmill exercise (Table 12).

Table 12: Correlation coefficient (P-value) between the same blood parameters before and after exercise and competition (n=12)

	aqua treadmill (Thursday)		competition	
	correlation	P-value	correlation	P-value
bilirubin (µmol/L)	**0.87**	<0.01	**0.56**	0.01
glucose (mmol/L)	0.17	0.59	0.25	0.24
lactate (mmol/L)	-0.47	0.12	0.47	0.02
triglyceride (mmol/L)	**0.74**	<0.01	0.37	0.07
cholesterol (mmol/L)	**0.63**	0.03	**0.79**	<0.01
LDH (U/L)	**0.76**	<0.01	**0.58**	0.01
AST (U/L)	**0.82**	<0.01	**0.98**	<0.01
CK (U/L)	**0.77**	<0.01	**0.92**	<0.01
cortisol (nmol/L)	**0.64**	0.02	**0.56**	<0.01

The blood samples were taken on Thursday before and after the aqua treadmill training, and both days of the event (Saturday, Sunday) morning and immediately after the first course.

Other researchers observed highest correlation with blood lactate concentration 2 and 5 minutes after exercise on treadmill (Evans et al., 1993), in our results the lactate level before and after training did not correlate significantly. This may be due to the lactate transport activity, Standardbred horses can be divided into two populations: one with high and the other with low lactate transport activity in their RBC (Vaihkönen and Pösö, 1998). Lactate transport capacity appears to be inherited, with the high capacity being caused by the dominant allele (Vaihkönen et al., 2002).

Rumley et al. (1985) demonstrated total CK did not correlate with finishing time at 30 minutes or 30 hours post race. However, in our study the CK activity very closely correlated in both aqua treadmill and competition basis before exercise and after exercise. The most of the blood parameters has significant correlation before and after competition or exercise. This information indicates that evaluating the effect of exercise on blood biochemical parameters can not be judged without the knowledge of baseline level. Furthermore, the response given to a workload is depends on the baseline value of the blood parameter. Therefore, when evaluating exercise induced changes in blood parameters it can be suggested that the baseline level should be used as a covariate to reduce the effect of individual variation. We have tested that hypothesis on our dataset and the results are presented in Table 13.

Our results clearly demonstrate that in the case of horses using the baseline level as a covariate makes individual effect non-significant in most of the cases. The exception of LDH and cortisol indicates that other factors than baseline level affects considerable the individual response. Waguespack et al. (2011) tested the usefulness of baseline plasma urea level as covariate on the number of detected significant differences in pigs

throughout several experiments. In their case using the baseline level did not resulted in noteworthy increase in the number of significant differences. However in the case of pigs the baseline level was a value measured at the beginning of the trial and the effect of nutritional treatment was measured several weeks later. Therefore, it is obvious that there is a weak correlation between the two values. However in the case of horse studies there is a relative short time difference between the baseline and test measurements. Since in our study there was a considerable difference between post exercise blood parameters of aqua training and show jumping competition, it had no meaning to test that theory. However, in evaluating the effects of treatment using the same type of workload, this approach can be useful.

Table 13: The effect of baseline level used as a covariate on the significance of individual differences in blood parameters (n=12)

Parameter	Horse				Model1	Model2	
	A	B	C	D	H	H	BC
Bilirubin (µmol/L)	16.1^{ab}	$17.7^{a,b}$	18.3^{a}	14.4^{b}	*	NS	**
Glucose (mmol/L)	4.2	4.5	4.2	4.4	NS	NS	+
Lactate (mmol/L)	1.2^{b}	$1.3^{a,b}$	2.9^{a}	$1.5^{a,b}$	*	NS	*
Triglyceride (mmol/L)	0.37^{b}	$0.47^{a,b}$	$0.45^{a,b}$	0.48^{a}	*	+	***
Cholesterol (mmol/L)	2.3^{a}	2.3^{a}	1.9^{b}	2.1^{b}	***	NS	+
LDH (U/L)	781^{a}	629^{b}	622^{b}	$742^{a,b}$	**	*	*
AST (U/L)	302^{b}	287^{b}	426^{a}	325^{b}	***	NS	***
CK (U/L)	198^{b}	230^{b}	323^{a}	268^{ab}	***	NS	***
Cortisol (nmol/L)	158^{b}	173^{b}	252^{a}	176^{b}	***	***	***

^{a,b,c} Means in a row lacking a common superscript differ ($P < 0.05$) (calculated in model 1); H – horse; BC – baseline as covariate; NS – not significant; * - $P<0.05$; ** - $P<0.01$; *** - $P<0.001$

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

5.3.1. 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. Increasing the maximum speed of the aqua treadmill had no influence on the heart rate average of horses subjected to the training (Table 14). Intensive exercise causes an increase in plasma biochemical markers (Volfinger et al., 1994). Elevated activities of AST and CK can also be associated with muscle damage and/or muscular damage (Balogh et al., 2001). The increasing phase can be explained by the increase of anaerobic threshold. The higher response of glycolytic metabolism can cause permeability changes in muscle fibre membranes (Harris et al., 1990). However, if anaerobic threshold value stabilizes and adaptation occurs, which can result in decreased AST and CK activities. This theory is supported by the results of Fazio et al. (2011), who observed reduction in AST and CK activities after an increasing phase at the end of a prolonged exercise period (80 days). In our results the 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 (cholesterol, bilirubin) to those observed at the

lowest training intensity. Training of horses on dry or low water level (< 77% of withers height) treadmill increases linearly the lactate accumulation in plasma (Galloux et al., 1995; Lindner et al., 2010, 2012) at low work intensities. However, experimental results (Lindner et al., 2012) demonstrated that lactate level was decreased when the level of water was increased from 63 to 77% of the withers height. In our study the level of water was adjusted about 85% of the withers height (deep water treadmill) from the beginning of the exercise test, and the plasma lactate level decreased even in spite of training (Table 14). Deep water aqua training (where the level of water is around 80% of the withers height) results in lower body temperature (Nankervis et al., 2008). It is demonstrated that low temperature water (13-16 °C vs 19 °C) can reduce even the heart rate response to treadmill exercise (Nankervis et al., 2008). The temperature of water in our study was 21 °C, which is still well beneath the normal body temperature. Thus, the cooling effect of water on muscles was certainly present in our case as well. Therefore, it can be suspected that this effect is behind the unusual lactate response compared to dry or low water level treadmill training. This is supported by the results of Kang et al. (2012), where the slow (3.6 km/h) swimming training of riding horses resulted in significantly lower heart rate, glucose and lactate concentration. The importance of this phenomenon is that it is common in equine conditioning studies to use the relationship between blood lactate and exercise speed like V_{LA4} (speed at lactate level reaches the 4 mmol/L value) to define exercise intensity to give adequate stimulus to improve performance (Trilk et al., 2002). Since aqua training attenuated the effect of exercise on lactate production, this value cannot be correctly calculated. The question still remains, whether the energy metabolism of muscles was really altered. Low lactate accumulation is the

sign of aerobic energy metabolism and the use of slow contracting Type I muscle fibres. Plasma glucose and free fatty acids are the most important energy sources in submaximal exercise (Lawrence, 1994). Lowered plasma glucose level indicates it's more intensive utilisation, while elevated triglyceride level shows the increased mobilisation of fat stores. These data indicate that aerobic energy generation was more intensive during aqua training. In our study 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 value decreased to an even lower level measured at the lowest intensity aqua training. Several studies have shown (Kedzierski and Podolak, 2002; Muñoz et al., 2002; Kędzierski et al., 2009) that high-intensity exercise may result higher triglyceride level which is reduced as the effect of several weeks of regular training. The decreasing triglyceride value of the highest intensity (13 km/h) indicates that the skeletal muscles due to the regular high-intensity exercise (result adaptation process) cause higher oxidative capacity and increased capacity of fats (triglycerides) as an energy source. Show jumping competition including warming up and riding the course is about a 40 to 50 minutes moderately intensive work (maximum speed is about 24 km/h), and it falls into the aerobic energy supply range (Ellis and Hill, 2005). Therefore it can be hypothesised that deep water aqua training can improve the fitness of show jumpers.

Table 14: Effect of aqua training intensity on the heart rate and plasma biochemical parameters of show jumpers during and after aqua training (n=4/treatment)

Plasma parameter	Aqua training intensity (max. speed of treadmill, km/h)			Time of sampling (min after starting aqua training)						RMSE	P int.	P samp.	P cov.
	9	11	13	0	10	40	44	60	120				
Heart rate (bpm)	76.3	76.5	71.7	55.4^{c}	79.9^{b}	107.7^{a}	56.3^{c}	-	-	8.9	ns	***	***
Lactate (mmol/L)	0.66^{a}	0.58^{ab}	0.56^{b}	0.63^{b}	0.41^{c}	0.38^{c}	0.40^{c}	0.68^{b}	1.11^{a}	0.14	*	***	*
AST (U/L)	307^{a}	279^{b}	305^{a}	308	298	299	294	290	293	30.0	**	ns	***
CK (U/L)	220^{a}	184^{b}	231^{a}	232	217	208	205	200	208	28.6	***	ns	***
LDH (U/L)	672^{a}	543^{b}	655^{a}	668	635	609	611	603	613	60.9	***	ns	***
Glucose (mmol/L)	4.21^{b}	4.49^{a}	4.52^{a}	4.80^{b}	3.93^{d}	3.93^{d}	3.99^{d}	4.38^{c}	5.41^{a}	0.44	*	***	**
Triglyceride (mmol/L)	0.37^{b}	0.44^{a}	0.33^{c}	0.36^{bc}	0.36^{bc}	0.43^{a}	0.44^{a}	0.38^{ab}	0.31^{c}	0.07	***	***	***
Cholesterol (mmol/L)	1.98^{b}	1.85^{c}	2.10^{a}	2.07	2.00	1.97	1.96	1.97	1.91	0.16	***	ns	***
Cortisol (nmol/L)	191^{a}	154^{b}	207^{a}	155^{c}	183^{b}	219^{a}	216^{a}	200^{ab}	132^{c}	33.1	***	***	***
Bilirubin (μmol/L)	16.3^{a}	12.7^{b}	18.1^{c}	15.0	14.9	16.0	15.9	16.4	15.9	1.56	***	ns	***

^{a,b,c,d} Means with the same letter are not significantly different; ns – not significant; * - P<0.05; ** - P<0.01; *** - P<0.001
RMSE – root mean square error; P int. – probability of the effect of aqua training intensity; P samp. – probability of the effect of sampling time; P cov. – probability of the effect of covariate (parameter value at rest)

5.3.2. *Competition*

It is proven that the effect of training should be monitored in competition environment as well (Fazio et al., 2011). Heart rate is in close relationship with oxygen uptake and energy expenditure during exercise (Coenen, 2005). Good performing horses are having a quicker recovery after exercise bout (Bitschnau et al., 2010). Covalesky et al. (1992) measured similar post class heart rate in show jumpers competing at similar heights, compared to our results (Table 15). However, horses competing at higher classes had lower post performance heart rate values (Covalesky et al., 1992). Higher heart rate can be associated with higher lactate concentrations, more faults, lower technical score and closer take-off distance (Harris et al., 2014). These results indicate that heart rate measured after show jumping can reflect the differences in fitness. In our experiment the medium level aqua training (maximum speed 11 km/h) reduced the heart rate measured after show jumping course, indicating that the training resulted in improvement of fitness. Further increase in the training intensity had no effect on heart rate.

The unaffected lactate level indicate that the anaerobic capacity was not affected by aqua training. Values are around the anaerobic threshold value (2-2.25 mmol/L; Spurway, 1992; Valette et al., 1993), suggesting that the energy requirements of the performance was mainly met by aerobic energy sources. Interestingly, show jumpers competing on similar or even lower obstacle height developed higher lactate concentrations (3.6-3.9 mmol/L; Covalesky et al., 1992; Sloet van Oldruitenborgh-Oosterbaan et al., 2006). In our study the lactate level was unchanged by aqua training, however the measured values were about two times higher compared to those experienced after one hour the aqua training. The minimum speed of the horses was similarly 350 m/min, but the course length was 700 m.

In practice show jumping courses including 12 obstacles (13-15 efforts) rarely exceed 500 m in length. These results show that the length of the course can significantly affect the plasma lactate concentration. Therefore for conclusions applicable to practice field tests should run under practical conditions.

Activities of AST, CK, LDH and levels 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. The elevated activities of AST, CK and LDH after even at lower speed training indicates that deep water aqua training is a strenuous exercise to horses. Excessive training does not necessarily result in an increase of CK activity (Harris et al., 1997; Hamlin et al., 2002). Considerably lower CK activity was measured in show jumpers performing in higher class competitions (Art et al., 1990[ab]). Nevertheless, the role of AST and CK in signalling muscle damage is confirmed by the results that even a muscle biopsy can increase the enzyme activity (Soares et al., 2013). In our study competing on consecutive days resulted in elevated AST and CK activities. These results suggests that the aqua training causing an increased muscle damage at the initial phase of training, but then adaptation occurs.

The major energy sources for oxidation for muscle cells are free fatty acids and glucose via anaerobic glycolysis (Vervuert, 2011). Plasma glucose level remains stable during short and low-intensity exercise, but increases even in moderate intensity exercises with about 2-4 mmol/L (Hyyppä, 2008). Contrary to that in our cases we observed decreased glucose level after competition (Table 15) compared to the rest values

(5.06, 5.02, 4.93 and 5.09 mmol/L for control, 9- 11- and 13 km/h maximum aqua trained groups, respectively). The decreases for the aquatrained groups are about 0.5 mmol/L; while 1.4 mmol/L for the control group. Art et al. (1990[b]) demonstrated similar results with show jumpers. The decrease of plasma glucose level is due to the depletion of liver glycogen, which is demonstrated in endurance horses after a three to four hour prolonged low intensity exercise (Hyyppä, 2001). However, closely observing the figure it is clear that in the first half an hour of the exercise, the plasma glucose level is decreasing by about 1 mmol/L and the raising phase starts after that. The warm up period and the completion of the show jumping course takes about 30 to 40 minutes, which can explain why we and others found decreased plasma glucose level after show jumping test. Since aquatrained horses had lower decrease in plasma glucose, and increased plasma triglyceride it can be assumed that the oxidative energy regeneration capacity of horses is improved. Aqua training had no influence on the levels of plasma cholesterol, cortisol and bilirubin measured after competition. Similar cortisol values were found during treadmill test and show jumping course, which shows that the deep water aqua treadmill training and competition cause similar stress to the horses.

Table 15: Effect of aqua training intensity on the heart rate and plasma biochemical parameters of show jumpers after competition (n=4/treatment)

Plasma parameter	Aqua training intensity (max. speed of treadmill, km/h)				Days of competition		RMSE	P int.	P day	P cov.
	Control	9	11	13	Sat	Sun				
Heart rate (bpm)	89.3[a]	82.6[ab]	76.9[b]	75.4[b]	81.8	80.3	9.43	*	ns	**
Lactate (mmol/L)	2.31	2.77	2.42	2.03	2.31	2.46	0.99	ns	ns	***
AST (U/L)	327[c]	374[a]	356[b]	337[c]	341[b]	356[a]	15.6	***	**	***
CK (U/L)	221[c]	328[a]	245[bc]	267[b]	251[b]	279[a]	26.4	***	**	***
LDH (U/L)	633[b]	833[a]	676[b]	695[b]	686	732	94.2	**	ns	*
Glucose (mmol/L)	3.60[b]	4.51[a]	4.38[a]	4.60[a]	4.20	4.35	0.45	***	ns	ns
Triglyceride (mmol/L)	0.34[b]	0.45[a]	0.46[a]	0.43[a]	0.42	0.42	0.057	**	ns	*
Cholesterol (mmol/L)	2.30	2.14	2.31	2.26	2.22	2.28	0.16	ns	ns	***
Cortisol (nmol/L)	199	172	164	193	183	181	40.1	ns	ns	**
Bilirubin (μmol/L)	16.7	16.0	17.1	17.9	16.7	17.1	2.00	ns	ns	***

[a,b,c] Means with the same letter are not significantly different; * - P<0.05; ** - P<0.01; *** - P<0.001; Sat - Saturday, Sun - sunday; RMSE – root mean square error; P int. – probability of the effect of aqua training intensity; P day – probability of the effect of days of competition; P cov. – probability of the effect of covariate (parameter value at rest)

5.3.3. *Correlation between plasma parameters during aqua training and after competition*

Before the aqua treadmill training significant positive correlations (Table 16) were observed between AST - bilirubin, AST – triglycerides, AST – CK and CK – bilirubin blood plasma biochemical parameters. Similarly the correlation between these parameters was also significant or tended to be significant after the aqua training. Furthermore, significant correlations were found between many other parameters like bilirubin – cortisol, cholesterol – LDH, AST – cortisol and CK – cortisol. Release of cortisol allows an individual to tolerate and adapt to challenges to homeostasis that occur in every life (Willmore and Costill, 1994; Thornton, 1985). Cortisol is increased in the horse during a wide variety of exercise activity (Horohov et al., 1999; Hyyppä 2001; Snow and Rose, 1981).The release of cortisol in the horse appears to be affected by both intensity and duration of exercise (Thornton, 1985; Snow and MacKenzie, 1977). Cortisol level is closely correlated with CK and AST at both exercise and competition, so horses that are more susceptible to stress can have elevated CK and AST activities as well. Muscle (myocarium or/and striated muscle, or smooth muscle) damage or hepatocyte damage can also increase plasma AST activity (Stockham and Scott, 2002). Bilirubin is a pigment produced mostly from red blood cells. Bilirubin is elevated for generally two reasons. Increased bilirubin is noted if the horse is worked beyond what it is capable of, creating muscle damage and red cell damage too, or if there is a problem with the bile ducts or the liver (Ralston and Larson, 1989). So it is fully physiological that we found significant correlation between bilirubin and CK, AST.

The available energy source is important during exercise or competition. Glucose is the primary source of energy for somatic cells. Plasma glucose

concentration tends to decrease during prolonged exercise (>3 hours) but during short, intensive exercise both decrease and increase (Pösö and Hyyppä, 1999; Snow and MacKenzie, 1977) have been recorded, depending on the exercise intensity and training and feeding status of the horse. The regulation of blood glucose level during exercise is a complex phenomenon. We could not find a correlation between glucose and other blood parameters.

Table 16: Correlation between blood parameters before (BA) and after (AA) aqua training (n=12)

BA		bil	gluc	lac	trig	chol	LDH	AST	CK	cor	AA
	bil	1.00	-0.05 (0.87)	-0.05 (0.88)	0.22 (0.49)	0.35 (0.26)	0.44 (0.15)	**0.59** (0.05)	**0.82** (0.01)	**0.73** (0.01)	
	gluc	-0.17 (0.59)	1.00	0.22 (0.49)	0.12 (0.71)	0.02 (0.96)	-0.08 (0.79)	-0.30 (0.34)	-0.07 (0.83)	-0.08 (0.81)	
	lac	0.18 (0.58)	0.37 (0.23)	1.00	0.34 (0.28)	0.25 (0.44)	0.45 (0.15)	0.01 (0.99)	0.01 (0.96)	0.17 (0.59)	
	trig	0.11 (0.73)	0.15 (0.63)	0.27 (0.40)	1.00	0.06 (0.85)	-0.04 (0.91)	0.51 (0.09)	0.42 (0.17)	0.29 (0.34)	
	chol	0.11 (0.73)	-0.15 (0.63)	0.41 (0.19)	0.20 (0.52)	1.00	**0.79** (0.01)	-0.01 (0.98)	0.06 (0.84)	0.06 (0.85)	
	LDH	0.28 (0.38)	0.27 (0.39)	0.15 (0.63)	-0.01 (0.96)	0.38 (0.22)	1.00	0.28 (0.38)	0.30 (0.34)	0.42 (0.17)	
	AST	0.59 (0.04)	0.21 (0.52)	0.37 (0.24)	**0.68** (0.01)	-0.15 (0.63)	0.08 (0.81)	1.00	**0.79** (0.01)	**0.79** (0.01)	
	CK	**0.77** (0.01)	0.05 (0.87)	0.12 (0.71)	0.42 (0.17)	-0.19 (0.56)	0.09 (0.79)	**0.86** (0.01)	1.00	**0.92** (<0.01)	
	cor	0.39 (0.20)	0.24 (0.44)	-0.17 (0.59)	0.05 (0.87)	0.06 (0.84)	0.19 (0.55)	0.22 (0.49)	0.51 (0.09)	1.00	

bil=bilirubin; gluc=glucose; lac=lactate; trig=triglyceride; chol=cholesterol; cor=cortisol; strong of correlation (P-value)
The blood samples were taken on Thursday before and after the aqua treadmill training

We observed both before and after competition significantly positive and negative correlation between blood plasma parameters (Table 17). Cholesterol showed significantly negative correlation with: AST, CK, cortisol (before competition) and AST, CK, lactate and cortisol (after competition). We are aware that the cholesterol level decreases as the effect of exercise (Lopez et al., 1974), but the significant negative correlation between cholesterol and CK, AST or cortisol can not be explained. We have to note, that when muscle damage occurs, HDL fraction immediately increases to maintain membrane homeostasis. Therefore, measuring total cholesterol only is less informative.

Table 17: Correlation between blood parameters before (BC) and after (AC) competition (n=12)

BC		bil	gluc	lac	trig	chol	LDH	AST	CK	Cor	AC
	bil	1.00	0.15 (0.48)	0.15 (0.49)	0.19 (0.38)	0.23 (0.28)	-0.29 (0.16)	0.27 (0.19)	0.13 (0.54)	0.47 (0.02)	
	gluc	0.17 (0.43)	1.00	-0.21 (0.33)	0.24 (0.26)	-0.03 (0.89)	-0.19 (0.38)	-0.12 (0.59)	0.08 (0.70)	-0.04 (0.84)	
	lac	**0.53** (0.01)	0.08 (0.69)	1.00	0.01 (0.97)	**-0.63** (0.01)	-0.19 (0.38)	**0.88** (<0.01)	**0.67** (0.01)	**0.81** (<0.01)	
	trig	0.25 (0.02)	-0.24 (0.25)	0.15 (0.47)	1.00	0.09 (0.67)	-0.13 (0.55)	-0.09 (0.66)	0.13 (0.54)	-0.01 (0.95)	
	chol	-0.09 (0.66)	0.41 (0.04)	-0.38 (0.06)	-0.29 (0.17)	1.00	0.18 (0.39)	**-0.52** (0.01)	**-0.59** (<0.01)	**-0.49** (0.01)	
	LDH	**0.58** (0.01)	0.01 (0.99)	0.04 (0.85)	0.29 (0.17)	0.08 (0.71)	1.00	-0.02 (0.93)	0.18 (0.40)	-0.42 (0.04)	
	AST	0.19 (0.37)	-0.32 (0.13)	0.48 (0.02)	0.08 (0.71)	**-0.63** (0.01)	0.13 (0.54)	1.00	**0.76** (<0.01)	**0.69** (<0.01)	
	CK	0.11 (0.62)	-0.28 (0.19)	0.32 (0.14)	0.14 (0.51)	**-0.67** (0.01)	0.22 (0.31)	**0.86** (<0.01)	1.00	**0.51** (0.01)	
	Cor	0.31 (0.14)	0.08 (0.71)	0.23 (0.27)	-0.07 (0.73)	**-0.50** (0.01)	0.37 (0.37)	**0.63** (0.01)	**0.68** (0.01)	1.00	

bil=bilirubin; gluc=glucose; lac=lactate; trig=triglyceride; chol=cholesterol; cor=cortisol; strong of correlation (P-value)
blood samples were taken both days of the event (Saturday, Sunday) before and immediately after the first course.

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). Tateo et al. (2008) demonstrated that there was no correlation between the method of conditioning and the activities of CK, LDH or AST in the field test in Italian Standardbred horses. In all cases we found positive correlation between CK and AST (before and after aqua treadmill, before and after competition). CK and AST enzyme activates are increased when the muscle or the heart muscle is compromised. Usually this is used to detect damage to the heart muscle or skeletal muscle. Particularly strong correlation can be found between lactate and AST, CK and AST after competition. Plasma enzymes activities (AST, CK, and LDH) are increased typically in horses during jumping or endurance exercise (Balogh et al., 2001; Krywanek et al., 1996). Lactate dehydrogenase (LDH) is a cytoplasm enzyme that catalyses the conversion of pyruvate to lactate at the end of glycolysis, when oxygen is absent or in short supply. At high concentrations of lactate, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased. The acid form of lactate (lactic acid) is produced by various cells of the body. For instance during intense exercise, the muscle cells produce lactic acid, which diffuses out from the muscle cells into the blood where it can be measured. Accumulation of lactic acid occurs when there is a high demand for energy but the supply of oxygen to the cells is limited, such as what happens during anaerobic exercise.

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 (Table 18).

The majority of trainers use empirical training programs to train a horse in a specific discipline. The assessment of fitness and improvement of fitness usually depends on subjective evaluation of trainer/rider (Fraipont et al., 2012). One of the main shortages of experiments with different conditioning programs is that none or not well documented comparisons were made to other conditioning programmes (Werkmann et al., 1996). Furthermore, studies do not check the performance in competition situation after different condition programmes. The finding of markers for testing the effectiveness of training programs has a high importance. Some studies indicate that the thoroughbred horses having superior competition performance have lower heart rate during trotting and slow gallops and lower lactate level after treadmill exercise test (Evans et al., 1993). As fitness increases, the post ride lactate level decreases in case of endurance horses (Lindner, 2010; Catherine, 2008). In case of show jumpers the differences in the level of performance in the same class are hardly can be determined correctly. The penalty points at the same class do not reflect in blood biochemical parameters measured after competition due to the fact that numerous other factors such as neuromuscular coordination, experience, motivation, respect of the fence, the rider's skill and the warming up process have influence (Art et al., 1990[b]). However, when the penalty points are examined over several shows, good correlation (R=0.75) can be found between the results and the blood plasma lactate level. Together with the existing individual differences it is indicating that the various training resulted in different level of fitness. Lactate level measured after competition had weak positive correlation only with plasma values obtained one hour after aqua

training (Table 18). AST, CK and LDH activities had weak to moderate correlation, regardless to sampling time. 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. In our studies the weak to moderate correlation between some prior and post training plasma biochemical values and post competition levels indicate that these markers are not robust enough to predict fitness to compete.

Table 18: Correlation of plasma biochemical parameters measured after competition with values determined before, after and one hour after aqua training (n=12)

correlation with values measured:	Plasma biochemical parameters								
	Lactate	AST	CK	LDH	Glucose	Triglyceride	Cholesterol	Cortisol	Bilirubin
before aqua training (T0)	0.18	0.68***	0.73***	0.52**	-0.37	0.21	0.45*	0.10	0.41*
after aqua training (T4)	-0.17	0.54**	0.41*	0.51*	-0.24	0.19	0.12	0.55**	0.12
one hour after aqua training (T5)	0.52**	0.61**	0.79***	0.64***	-0.02	0.15	0.11	0.52**	0.18

* - P<0.05; ** - P<0.01; *** - P<0.00

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

The measured lactate values (Figure 7) 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 level (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). Similarly to other studies (Hevesi et al., 2009; Vincze et al., 2012), plasma lactate level 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., 2008), and according with these results, based on the evolution of lactate level, it can be concluded that the temperature of the water (21 °C - reduces the production of the lactic acid through anaerobic glycolysis) and the perfusion of both flexor and extensor muscles led to the given experience. The thermoneutral zone in water for horses has not been established yet (Lindner et al., 2012), but in humans, this value ranges from 33 to 35 °C (Choukroun and Varene 1990). These values are likely to be lower in horses (Nankervis et al., 2008), 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 on the level of the water as well.

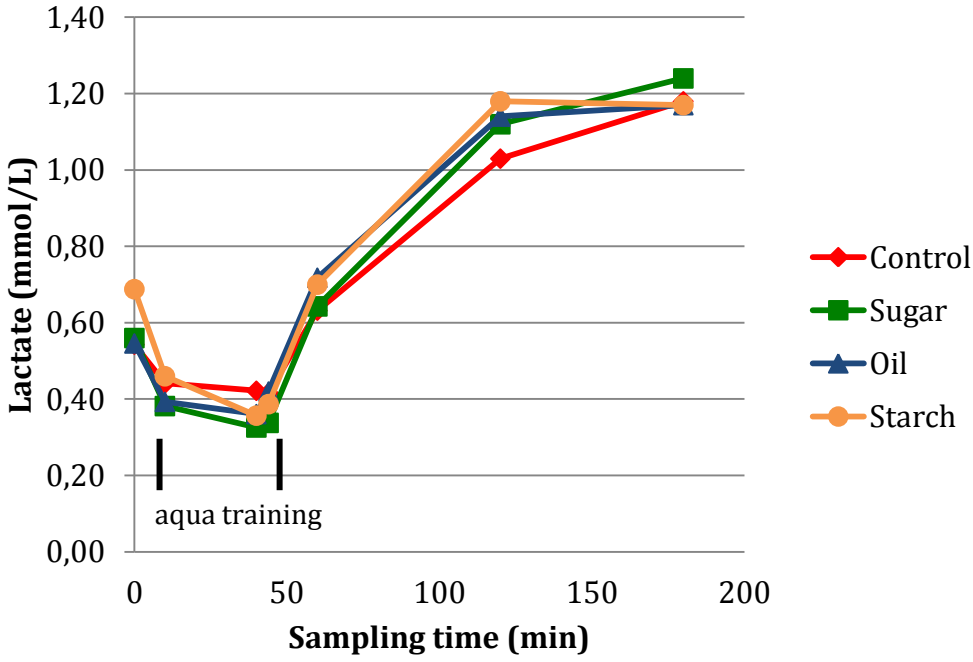

Figure 7: Effect of dietary main energy source on the plasma lactate level of equine athletes during and after deep water aqua treadmill training (n=4/treatments)

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 7). 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 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 activity at the 10th minute of high level aqua training (at the end of the first walking section) (Table 19). This result appeared as a tendency (P≤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 19). Interestingly, significantly lower CK activity was measured in show jumpers performing in higher class competitions (Art et al., 1990[ab]). 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 activity 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 activity (about 200-300 U/L). Only prolonged endurance exercise (60 km or more) can result in very high activity of CK (1000 – 30000 U/L) (Kerr and 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 10000 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.

Table 19: Effect of dietary main energy source on the plasma creatine kinase level (U/L) of equine athletes during and after deep water aqua treadmill training (n=4/treatment)

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 (T5)	44 (T6)	60 (T7)	120 (T8)	180 (T9)
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

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

Horses having sunflower oil as a main energy source in their concentrate had higher aspartate aminotransferase activity after two hours of the aqua training (Table 20).

Table 20: Effect of dietary main energy source on the plasma aspartate aminotransferase level (U/L) of equine athletes during and after deep water aqua treadmill training (n=4/treatment)

Gait	Stand (prepara-tion)	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 (T5)	44 (T6)	60 (T7)	120 (T8)	180 (T9)
Control	274	277	272	266	262	280	274[ab]
Sugar	272	277	277	280	274	283	275[ab]
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

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

This difference appears as a tendency at the end of the walking sections as well. The measured activity 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 activity were observed in higher class show jumping horses (Art et al., 1990[ab]) compared to our results. However, in English Thoroughbred horses with good racing

results continuously elevated (around 300 U/L) AST activity were found (Harris et al., 1990). Oliveira et al. (2014) measured AST activity well above 300 U/L in eventing horses tested on treadmill. Most of our measured AST activity are close to the 300 U/L activity. As also AST is mainly released from muscle, the simultaneously elevated AST and CK activities 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 21). 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 basis, the blood glucose and insulin levels decrease (Pagan et al., 1995; Stull et al., 1987). Lower glucose level was 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 level compared to the corn oil fed group (O'Connor et al., 2001). Further studies are needed to identify, which fatty acids are responsible for the altered glucose metabolism.

Table 21: Effect of dietary main energy source on the plasma glucose level (mmol/L) of equine athletes during and after deep water aqua treadmill training (n=4/treatment)

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 (T5)	44 (T6)	60 (T7)	120 (T8)	180 (T9)
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

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 22); while one hour after the training it was significantly lower compared to the other treatment groups. This can be explained that the control feed crude fat content and within this real fat content was quite

low. Thus, the result of oil supplement the body is likely to be better adapted to use fat as an energy source. 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), it is assumed that the racehorses exhibited an increased rate of hepatic synthesis of triglycerides (Pösö et al., 1989). This explanation is also supported by the result that triglyceride concentration increases during exercise as a function of the exercise intensity (Pösö and Hyyppä, 1999).

Table 22: Effect of dietary main energy source on the plasma triglyceride level (mmol/L) of equine athletes during and after deep water aqua treadmill training (n=4/treatment)

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 (T5)	44 (T6)	60 (T7)	120 (T8)	180 (T9)
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

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

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., 2008; Treiber et al., 2006). We believe that this adaptation was reflected in the lower plasma triglyceride level of the fat supplemented group.

6. 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 and the increased blood perfusion of the muscles 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.

7. 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.

8. SUMMARY

Physical training in the horse can cause several physiological and biochemical adaptations. Some of these changes can be measured by testing the biochemical parameters of blood. Several blood biochemical parameters are affected, such as glucose, lactate, cholesterol, triglyceride, lactate dehydrogenase (LDH) creatine kinase (CK), aspartate amino transferase (AST), bilirubin and cortisol. The most frequently analysed parameter is lactate. Lactate level can vary in a wide range, which depends on the intensity, duration and type of exercise. In most cases the blood enzymes activities (CK, LDH, AST) also increased after the various exercise bouts. The plasma glucose level can decrease and also increase depending on the training applied. Therefore, it can be concluded that for the more in depth understanding of exercise induced physiological changes, testing several biochemical parameters is necessary.

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.

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

Fitness is a certain metabolic status – as a result of training - which makes the equine athletes capable of good results in sport. Assess the fitness status in field conditions is therefore very important. Rather limited information is available on low class show jumpers, therefore the aim of this trial was study the effect of age and event on the biochemical blood parameters. During the winter indoor competition tournament (from October to February) blood samples were collected right after the first class of the last day of the event from all together fifteen show jumpers (five, six and seven years old horses, five randomly selected horse from each year category at same horses of the first and at the last competition of the tournament). From the blood plasma samples activities of LDH, CK, AST, and lactate, glucose, total cholesterol, triglyceride, total bilirubin and cortisol levels were measured for evaluating the training status of the horses. Five year old horses had significantly lower lactate level compared to six and seven year old horses. At the end of the tournament horses had lower glucose and higher cortisol level in the plasma right after show jumping class. The frequent stress situation can

elevate the cortisol response given. It can be concluded, that 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. Multivariate methods applied on several post exercise blood parameter can be useful to detect slight differences in fitness.

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

Studies with horses indicated that the responses of blood biochemical parameters to different exercises vary. Some study also indicated that significant individual variance exist in blood parameters, which makes difficult to detect treatment effect. Therefore, the aim of this study was to examine the correlation between plasma biochemical parameters of show jumpers before and after of high level aqua training and jumping course completion, and the effect of using baseline level as a covariate on the significance of horse effect. Four normally trained show jumpers ageing from 6 to 11 years were trained with high-intensity aqua treadmill in three periods during three days and after they did compete in the Indoor Show Jumping Championship. Blood samples were taken before and immediately after aqua treadmill training program and both days of the competition before and immediately after the course. From the blood plasma samples were determined the same parameters as in the first experiment. 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 exercise and competition in bilirubin, cholesterol, LDH, AST, 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. In conclusion when evaluating fitness of horses the type of exercise should be considered and biochemical values measured at rest should be used as covariate factor.

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

Aqua treadmill is mainly used for rehabilitation purposes, but research indicates that this equipment could be used for training as well. The few studies performed with aqua treadmill mainly followed lactate and heart rate changes. Therefore, the aim of this study was to test the effect of increasing aqua treadmill training intensity on the heart rate and several plasma biochemical parameters of show jumpers after competition. Four similarly trained show jumper horse competing at the same level (110 cm) were selected with age between 6 to 11 years. Blood samples (4 ml) were taken on the completion of the show jumping course after conventional training and after aqua training of varying intensity (9, 11, 13 km/h). Further blood samples were taken during the aqua treadmill training program as well. From the blood plasma samples were determined the same parameters as in the first experiment. 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.

The levels of glucose and triglyceride increased when the maximum speed was changed from 9 to 11 km/h. 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. 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. 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. 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 values. Cortisol level found after and one hour after aqua training did show weak correlation to values obtained after competition. Our results indicate that

the deep water aqua training alters the biochemical processes and can improve the aerobic energy supply of show jumpers. Aqua training is a strenuous exercise inducing increased muscle damage initially, but later adaptation occurs. Aqua training can improve the fitness even of lower class show jumpers.

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

A proper energy supply has a primary importance for the equine athlete. The source of energy has an influence on health, metabolism and sport performance. Therefore, the preference of energy sources depends on the type, intensity and length of the workload. 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 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 from the blood plasma samples were determined activities of LDH, CK, AST and levels of lactate, glucose and triglyceride. 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 ($P \leq 0.1$). 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. The elevated activities 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.

9. ÖSSZEFOGLALÁS

Az edzések hatására számos fiziológiai és biokémia változás figyelhető meg a lovak esetében is, amelyek a vér biokémiai paramétereinek mérésével - glükóz, laktát, koleszterin, triglicerid, laktát-dehidrogenáz (LDH) kreatin kináz (CK), aszpartát-amino-transzferáz (AST), bilirubin, kortizol - kimutathatóak. A szakirodalomban leggyakrabban vizsgált paraméter a laktát, aminek az értéke az edzés intenzitásától, időtartalmától, típusától függően igen széles tartományban mozoghat. A különböző típusú edzések hatására a vérplazma enzimek (CK, LDH, AST) aktivitása általában emelkedik, míg a vércukorszint csökkenhet és emelkedhet egyaránt. Az edzések által kiváltott fiziológiai változások jobb megértése érdekében a vér több biokémiai paramétereinek a vizsgálata szükséges egyidejűleg.

A kutatás fő céljai a következők voltak:

- Az életkor és a verseny időpont hatásának vizsgálata a díjugrató lovak vérplazmájának verseny után mért biokémiai paramétereire és enzim aktivitására.

- A mélyvizes futópad edzés intenzitásának hatása a szívverésszámra és néhány biokémiai plazmaparaméterre díjugrató lovak mélyvizes futópad edzése során és versenyt követően.

- A díjugrató lovak biokémiai vérparaméterei közötti összefüggések vizsgálata mélyvizes futópad edzés előtt, alatt és azt követően, valamint díjugrató versenyek alkalmával.

- A díjugrató lovak biokémiai vérparamétereinek vizsgálata különböző fő energiaforrású abrak etetése esetén mélyvizes futópad edzés előtt, alatt és azt követően.

Az életkor és a verseny hatásának vizsgálata díjugrató lovak vérparamétereire versenyt követően (1. kísérlet)

Az edzettség az edzés hatására létrejövő fizikai állapot, amely lovak esetében is hozzájárul a jobb sportteljesítmény eléréséhez, ezért fontos az aktuális edzettségi állapot szakszerű megállapítása. Viszonylag kevés irodalmi adat áll rendelkezésre az alacsonyabb szinteken versenyző díjugrató lovakról, ezért ennek a vizsgálatnak a célja az életkor és a verseny időpont hatásának vizsgálata a díjugrató lovak vérplazmájának verseny után mért biokémiai paramétereire és enzim aktivitására. A téli fedett pályás díjugrató versenysorozat során (októbertől februárig tart) tizenöt lótól (öt lovat választottunk ki véletlenszerűen az öt, hat és hét éves lovak versenyszámában induló lovak közül, melyeket a versenysorozat első és utolsó versenyén vizsgáltunk) közvetlenül az utolsó versenynap első pályája után vérmintát vettünk. A vérplazma mintákból meghatározásra került az LDH, CK, és AST aktivitás, a laktát, glükóz, összkoleszterin, triglicerid, összbilirubin és kortizol szint. Az ötéves lovak esetében szignifikánsan alacsonyabb laktát szintet mértünk a hat és hét éves lovakhoz viszonyítva. A versenysorozat utolsó versenyén szignifikánsan alacsonyabb glükóz és szignifikánsan magasabb kortizol szintet mértünk a versenysorozat első versenyéhez képest. A kortizol szint

emelkedését a gyakori stresszhelyzet okozhatta. Arra a következtetésre jutottunk, hogy a maximum 100 cm magasságú akadályokból álló pálya 300 m/perc irammal teljesítve nem jár anaerob energianyeréssel. A többváltozós módszerek alkalmazása az edzés utáni vérparaméterek vizsgálatában az edzettségben megmutatkozó kisebb különbségeket is képes megmutatni.

Az edzés típusának és a nyugalmi érték kovarianciájának hatása a díjugrató lovak vérplazma biokémiai paramétereire (2. kísérlet)

Számos tanulmány igazolta, hogy a különböző típusú edzések a vér biokémiai paramétereiben eltérő változásokat okoznak. Néhány tanulmány eredményei azt mutatták, hogy a vér biokémiai paramétereiben egyedi eltérések figyelhetők meg, ami megnehezítheti a kezelés hatás kimutatását. A vizsgálat célja, hogy kimutassuk a vér biokémiai paraméterei közötti összefüggéseket díjugrató lovak esetében mélyvízes futópad edzés előtt és azt követően, valamint díjugrató versenyen, továbbá, hogy a nyugalmi értéket, mint kovariáns faktor vizsgáljuk. Négy hagyományosan edzett díjugrató lovat (6-11 év) három periódusban mélyvízes futópadon edzettünk és díjugrató versenyen indítottunk. Vérmintákat vettünk a mélyvízes futópad edzések előtt és azt követően, valamint a díjugartó versenyen mindkét versenynap reggelén és az első pálya teljesítését követően azonnal. A vérmintákból ugyanazokat a vérparamétereket határoztuk meg, mint az első kísérlet esetében. A mélyvizes futópad edzés csak a glükóz, triglicerid és a kortizol értékek esetében okozott szignifikáns változást, míg a díjugrató verseny az AST kivételével az összes mért paraméterben szignifikánsan magasabb értéket eredményezett. Pozitív korrelációt találtunk a mélyvizes futópad edzés előtt és után, illetve a verseny előtt és után a bilirubin, koleszterin, LDH,

AST, CK és kortizol esetében. Ez az információ azt mutatja, hogy a vér biokémiai paraméterei alapján történő edzés hatást nem tudjuk helyesen megítélni a vérplazma paraméterek nyugalmi értékének ismerete nélkül. Eredményeink azt mutatják, hogy ha a kezdeti értéket, mint kovariánst használjuk, akkor az egyedi különbségek csökkenthetők. Éppen ezért, ha az edzettséget a vér biokémiai paraméterei alapján értékeljük, akkor a vérparaméterek nyugalmi értékét, mint kovariánst érdemes használni az egyedi különbségek csökkentése érdekében.

A mélyvizes futópad edzés intenzitásának hatása a díjugrató lovak vérparamétereire (2. kísérlet)

Kutatási eredmények bizonyítják, hogy a mélyvizes futópad a lovak rehabilitációja mellett edzésre is jól alkalmazható. A szakirodalomban található kutatások a vizes edzések alkalmával elsősorban a vér laktát szintjét és a szívverésszám változását vizsgálják. Éppen ezért a kísérlet célja a mélyvizes futópad edzés intenzitásának vizsgálata a szívverésszámra és néhány biokémiai plazmaparaméterre díjugrató lovak mélyvizes futópad edzése során és versenyt követően. Négy azonos szinten versenyző (110 cm), hagyományosan edzett 6-11 év közötti díjugrató lovat vizsgáltunk. Vérmintát vettünk a hagyományosan edzett, illetve a különböző intenzitáson (9, 11, 13 km/h) végzett mélyvizes futópad edzés követő díjugrató versenyek alkalmával, valamint az egyes periódusokban a harmadik mélyvizes futópados edzésprogram alatt. A vérmintákból ugyanazokat a vérparamétereket határoztuk meg, mint az első kísérlet esetében. A laktát és glükóz szint szignifikánsan csökkent a mélyvizes futópad edzés alatt, majd emelkedett az edzést követően. Az ügetőszakasz végére a triglicerid érték emelkedett, majd egy órával az edzést követően érte el a nyugalmi szintet. A megnövekedett kortizol

szint azt mutatja, hogy a mélyvizes futópadon végzett edzés stressz helyzetet okoz a lovaknak. Az AST, CK, LDH, aktivitás és a koleszterin és bilirubin értékek nem változtak a mélyvizes futópad edzés során. A vérplazma glükóz és triglicerid értéke emelkedett, amikor a futópad sebességét 9-ről 11 km/h sebességre emeltük. A vérplazma laktát szintje csökkent, amikor a futópad sebességét a maximálisra emeltük. A futópad sebességének a növelése (9 km/h-ról 11 km/h-ra) az AST, CK, LDH aktivitás és a koleszterin, kortizol és bilirubin értékek csökkenését okozta, azonban az intenzitás további növelése (13 km/h) hasonló, vagy magasabb értékeket eredményezett a futópad legkisebb sebességéhez viszonyítva. A közepes intenzitású futópados edzés hatására a szívverésszám csökkent a díjugrató pályát követően mért értékek esetében a legkisebb intenzitáson végzett futópados edzéshez viszonyítva A futópad maximális sebességének növelése 9-ről 11 km/h-ra csökkentette az AST, CK és LDH aktivitást, azonban a glükóz és triglicerid értékekre nem volt hatással. A mélyvizes futópad edzés előtt szignifikánsan pozitív korrelációt állapítottunk meg az AST – bilirubin, AST – triglicerid, AST – CK és CK – bilirubin esetében. További szignifikánsan pozitív korrelációt állapítottunk meg a bilirubin – kortizol, koleszterin – LDH, AST – kortizol és CK – kortizol között. A verseny előtt és közvetlenül a verseny után vett vérminták biokémiai paraméterei között pozitív és negatív korrelációkat is találtunk. A koleszterin szignifikánsan negatív korrelációt mutatott az AST, CK és kortizol paraméterekkel a verseny előtt vett vérminta alapján, valamint az AST, CK, laktát és kortizol értékekkel a verseny után mért vérparaméterek tekintetében. Pozitív korrelációt találtunk a verseny előtt mért laktát – bilirubin, LDH – bilirubin, AST – laktát értékek között és a verseny után mért AST – laktát, CK – laktát, CK – AST, kortizol – laktát, kortizol – AST, kortizol

– CK paraméterek esetében. Szoros korrelációt állapítottunk meg a laktát és AST, valamint a CK és AST paraméterek között a verseny végén vett minták esetében. A koleszterin és bilirubin érték közepes korrelációt mutatott a nyugalmi időszakban. A kortizol szint a mélyvizes futópad edzés után, illetve egy órával az edzést követően közepes korrelációt mutatott a verseny végén mért értékekkel. Eredményeink azt mutatják, hogy a mélyvizes futópados tréning a biokémiai folyamatokat megváltoztatja és javíthatja a díjugrató lovak aerob energia ellátását. A mélyvizes edzés kezdetben megerőltető a lovak számára és megnövekedett izomkárosodással jár, amihez a későbbiekben jól alkalmazkodnak. A vizes tréning javíthatja az edzettséget még alacsonyabb szintű díjugrató lovak esetében is.

A különböző energiaforrású takarmány hatása a vér biokémiai paramétereire mélyvizes futópadon történő edzés során (3. kísérlet)

A megfelelő energiaellátás elsődleges fontosságú a sportlovak esetében, mert hatással van az egészségi állapotra, az anyagcserére és a sportteljesítményre egyaránt. A különböző típusú, intenzitású és időtartamú mozgásformák más-más energiaforrást igényelnek. Mindezek alapján feltételezhető, hogy a különböző energiaforrású takarmány eltérő változásokat okoz a vér biokémiai paramétereiben mélyvizes futópad edzés során. A vizsgálatot 4 díjugrató lóval végeztük latin négyzet elrendezésben. Valamennyi kezelésben a lovak azonos mennyiségű réti szénát kaptak, míg a négy eltérő abraktakarmány adagot (kontroll, keményítő zabból, olaj napraforgóból, melasz cukorrépából) úgy állítottuk össze, hogy a különböző energiaforrások mellett az emészthető energia felvétel azonos legyen. Egy kísérleti periódus 10 nap adaptációból és 4 nap tesztelési időszakból állt, amikor a lovak mélyvizes futópad

edzésen vettek részt. Vérmintát vettünk az utolsó mélyvizes futópad edzés előtt, közben és után. A vérmintákból meghatározásra került az LDH, CK, AST aktivitás és a laktát, glükóz és triglicerid szint. Az eltérő energiaforrású takarmányok a vérplazma laktátszintjében hasonló változásokat okoztak. A takarmányadagban nagyobb arányban keményítőt fogyasztó csoportnál szignifikánsan alacsonyabb CK aktivitást mértünk ($P<0,05$) a mélyvizes futópad edzés lépés szakaszának végén. Ez tendencia formájában a későbbi eredményeknél ($P\leq0,1$) is megfigyelhető volt. A napraforgó olajat fogyasztó csoportoknál magasabb AST aktivitást tapasztaltunk két órával a mélyvizes edzést követően. A plazma triglicerid értéke ezen csoportnál tendenciózusan csökkent a mélyvizes futópad edzés végére ($P<0,1$), majd szignifikánsan alacsonyabb értéket mutatott egy órával az edzést követően. Az emelkedett CK és AST aktivitás azt jelzi, hogy a laktát szint önmagában nem tükrözi helyesen a mélyvizes futópad edzés terhelését. A rendelkezésre álló különböző energiaforrás megváltoztatja a metabolikus válaszokat a mélyvizes edzés során, akkor is, ha ezek nem számottevőek.

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12. PUBLICATIONS DERIVED FROM THE THESIS

12.1. 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)

12.2. 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)

12.3. 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

13. OTHER PUBLICATION

13.1. 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.

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14. CURRICULUM VITAE

I was born in Szeged on 28 February 1982, and attended primary and secondary school in my town of birth. I obtained General Certificate of Education in the József Eötvös Secondary School in 2000.

I studied at the Kaposvár University from 2000 to 2006, as agricultural engineer and as pedagogies, so I have got two degrees of the university in 2005 and 2006. During the university years in 2004 I obtained the certificate of riding trainer.
In 2009 got admission to the Doctoral School of Animal Science at the Kaposvár University.

I have been working for the Pannon Equestrian Academy of Kaposvár University since 2006 as a riding trainer. In 2008 I completed the eventing judge course and in 2009 I have got second-class eventing judge qualification. In 2011 I obtained level II. registration of eventing trainer.
Since 2013, I have worked at the Department of Equine Therapy and Hippology and participated in the teaching of several subjects like: Basic knowledge of horseback riding I., II., III., Horse riding, Horse economies and Basic training for horse and rider. I had supervised or co-supervised a number of undergraduate theses and I regularly reviewed theses.

In 2007, I obtained intermediate-level type C language exam in technical German (agriculture), and I had successfully passed the state accredited English B2 oral language examination in 2011 and English B1 combined language examination in 2014.