

The effect of different conditioning regimes on the performance of endurance horses

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Summary

This research compared two endurance training methods under Malaysian climatic conditions. Two teams of 9 Arab horses, all with previous experience in endurance competition, either at 160 or 120 km, were trained following two different regimes. The eventual goal was for the horses to participate in a 120 km endurance race approximately three months after the onset of the training. During four standardised exercise tests (SET), consisting of a 35km long track, progress was monitored by measuring the heart rate, rectal temperature and various blood parameters before-, immediately after and 30 minutes after finishing the test ride. There were significant differences between the two teams in heart rate, rectal temperature, aspartate aminotransferase activity, plasma sodium- and chloride concentration, lactate concentration and white blood cell-, segmented neutrophil- and thrombocyte count. Because the horses in the two teams had a different background and followed a different training regime, no conclusions could be drawn on the efficiency of the trainings method. However, in the actual 120 km competition, there was no difference in the performance of horses from either team.

Samenvatting

Dit onderzoek vergeleek twee verschillende endurance trainings methoden, onder Maleise klimaat omstandigheden. Twee groepen van 9 Arabieren, die allemaal eerdere ervaring in endurance wedstrijden tot 160 of 120 km hadden, werden volgens twee verschillende trainingsregimes getraind. Het uiteindelijke doel was om de paarden te laten deelnemen aan een 120 km endurance race, ongeveer drie maanden na het begin van de training. Tijdens vier "standardised exercise tests" (SET), bestaande uit dezelfde 35km lange tocht, werd de progressie bijgehouden door middel van het meten van de hartslag, rectale temperatuur en verschillende bloedparameters voor-, direct na- en 30 minuten na het eindigen van de test rit. Er waren significante verschillen tussen de twee groepen voor de hartslag, rectale temperatuur, aspartaat aminotransferase activiteit, plasma natrium- en chloride concentratie, lactaat concentratie en witte bloedcel-, gesegmenteerde neutrofiel- en thrombocyten telling. Omdat de paarden in de twee teams een verschillende achtergrond hadden en verschillende trainingsregimes volgden, kon er geen conclusie worden getrokken met betrekking tot de efficiëntie van beide regimes. Hoe dan ook, in de eigenlijke 120 km wedstrijd was er geen verschil tussen de prestatie van paarden uit beide teams.

Introduction

Since the 1950's endurance riding has been a competitive sport and in 1982 it was approved as an official discipline by the 'Fédération Equestre Internationale' (FEI). In modern day competition, emphasis should be placed on the horses finishing in good condition, rather than just who finishes first. This explains the stringent rules that have been developed with regard to veterinary checks throughout the race. It can take years for a combination to be ready to compete in a 160 km ride. Endurance requires extensive preparation and a good understanding between horse and rider. If these goals are achieved, the wellbeing of the horse can be maintained at all times (www.fei.org). The main goal of any conditioning programme in athletic horses is to improve performance by inducing physiological changes within the animal's body. Equine skeletal muscles have

considerable potential to adapt during training and these adaptations have important physiological implications with respect to stamina, strength and speed (*Rivero 2007*). Several parameters that are associated with exercise capacity change short-term or long-term, depending on the type of training (*Rivero 2007*).

The aim of this study was to design a more efficient way of training endurance horses, by comparing two different training regimes and trying to determine which parameters have the greatest predictive value regarding the horse's performance.

Cardiovascular adaptations

Heart rate measurements have proven to be a reliable indicator of the metabolic status of endurance horses suggesting that veterinary examinations according to the official FEI rules are adequate for protecting the health and welfare of horses competing in endurance races (*Sloet 1991*).

The extent to which the horse can increase its cardiac output and muscle blood flow appears to represent its major adaptation for maximal aerobic performance. The apparently greater flow capacity of the equine muscle bed during maximal whole-body exercise, compared to humans, suggests that the extent to which the circulation can adapt at the central level is a limiting factor in performance but also implies a role for an increase in arteriolar capacitance/muscle perfusion as an appropriate response to intense endurance training (*Physick-Sheard 1985*). Heart rate measurements proved to be a reliable indicator of the metabolic status of endurance horses suggesting that veterinary examinations according to the official Fédération Equestre Internationale rules are adequate to protect the health and welfare of horses competing in endurance races. (*Sloet 1991*)

Musculoskeletal adaptations

Equine skeletal muscle has a considerable capacity for adaption during training and, overall, these adaptations have important functional implications that influence power generation (strength), resistance to fatigue (stamina) and velocity of muscle fibre shortening (speed) (*Rivero 2007*). Ideally, conditioning programmes for athletic horses should be aimed at the development of muscle properties that optimize the equilibrium among these three physiological traits to best suit the athletic discipline pursued (*Rivero 2007*).

The nature and magnitude of muscular responses to training depends on two critical teams of factors. The first includes factors affecting the basal status of the muscle, i.e. the breed, age, sex, athletic discipline, level of fitness and training history of the horse. The second team of factors are related to the nature and amount of the applied stimulus: type, intensity, duration and frequency of exercise, as well as the total duration (length) of the conditioning programme (*Rivero 2007*). Training augments the functional capacity of skeletal muscle, in part by altering the amount of the proteins required for muscle contraction and energy metabolism. Muscle protein content and quality depend on the integrity of the remodelling/repair process of muscle during endurance training, which involves removal of an old protein and replacement with a new one (*Leisson 2008*).

Therefore, it is necessary to include systematic recovery periods in the training process to prevent overtraining and to achieve performance improvement (*Bruin 1994, Lehmann 1997, Tyler-McGowan 1999*). Muscle adaptation will not occur if the training stimulus is given too frequently, because it will interrupt the recovery phase. Conversely, if the training period between training sessions is too long, overcompensation will result in regression to the original (i.e. non-trained) functional state, without any progressive improvements (*Bruin 1994, Seene 2004*).

Serum titres of creatine kinase (CK) and aspartate aminotransferase (AST) increase significantly 4-6 hours after an endurance race and remain elevated for at least 18-20 hours after the race (*Hess 2000*). Muscle damage, indicated by plasma CK and AST activity, could be a good performance indicator (*Gondim 2009, Williams 2005*). During a 210 km endurance race, *Gondim et al. (2009)* found that horses that were eliminated due to lameness or fatigue syndrome (estimated by an inadequate recovery index) showed significantly higher plasma CK levels compared to the horses that were still in good condition at the end of the race and *Williams et al. (2005)* found that horses that did not finish an 80 km ride had significantly higher CK and AST activities before, during and after the ride compared to horses that did (*Gondim 2009, Williams 2005*).

The adaptive response to training can follow two basic routes, although in practice they frequently occur simultaneously and are accompanied with changes in the microvasculature. The first is the quantitative response, which consists primarily of fibre hypertrophy, in which myofibres increase in size but retain their basal properties; this predominantly increases muscle strength. The second route is a qualitative response or remodelling (the most common response in endurance training), in which fibres acquire markedly different metabolic and structural characteristics, producing a muscle that is much more resistant to fatigue, but with an intrinsically decreased contraction velocity (i.e. speed) (*Rivero 2007*).

Adaptations that favour the stamina traits of a muscle the most, and are thus seen as a result of effective endurance training, are an increase in: capillary volume, mitochondrial density, aerobic muscle enzyme activity, glucose and fatty acid transport, triglycerides- and glycogen concentrations (as well as sparing of these substrates during exercise) and a decrease in post-exercise muscle lactate concentration (*Rivero 2007, Leisson 2008*).

Furthermore, endurance training promotes a transition from type II to type I muscle fibre, which happens at the expense of the type II fibre population (*Thayer 2000*).

Marked muscle hypertrophy in endurance horses can be achieved by exercise of higher intensity (80% of V_{Ia4}) and moderate-to-long-duration (60–80 min) for 12 weeks. However, the benefit of this adaptation to endurance activity is unclear (*Rivero 2007*). Long-term (6–8 months) endurance training programmes with exercise of low intensity (25–50% of V_{Ia4}) and long duration (60–120 min) result in a considerable increase in aerobic capacity, a moderate improvement in muscular strength (discrete hypertrophy) and reduced anaerobic capacity (*Rivero 2007, Leisson 2008*).

Blood cell changes

An increase in oxidative burst activity and phagocytosis in equine peripheral blood neutrophils has been associated with moderate intensity exercise, suggesting a possible beneficial effect on immunity (*Raidal 2000*). High intensity exercise and training however, appear to have a detrimental effect on neutrophil function that may indicate an impaired immune response, rendering the individual horse at an increased risk of developing an infectious disease (*Raidal 2000*). The production and circulating numbers of phagocytes are tightly regulated and controlled by various humoral factors, including colony-stimulating factors and interleukins. Consequently, the number of phagocytes in the blood reflects circumstances in the tissues, as well as the proliferative function of the bone marrow (*Merck Veterinary Manual Online*).

Splenic contractions, under the influence of catecholamines, are the main cause of changes in the erythrogram after race training, but exercise-induced fluid shifts also contribute to these changes (*Hinchcliff 2004*). These changes result in an increase in packed cell volume (PCV), erythrocyte count and haemoglobin concentration. The extent of the increase in PCV depends on the intensity of the exercise, with a linear relationship

to speed, with a maximum PCV of 60-65% (*Hinchcliff 2004*). Cywinska et al (2010) also found a clearly visible increase in PCV in response to exercise after all training sessions, that could reach up to 60% in individual cases (*Cywinska 2010*).

Intravascular haemolysis in athletic horses results from increased fragility of erythrocytes due to frequent accumulation in the spleen (*Hanzawa 1999, Hanzawa 2000*). Anaerobic exercise also promotes an increase in the osmotic fragility of red blood cells, while aerobic exertion causes the opposite effect. During anaerobic exercise, the fragility of erythrocytes increases progressively with running velocity (*Cywinska 2010*). Exercise induced erythrocyte fragility is also related to a decrease in blood pH resulting from increases in carbon dioxide partial pressure and lactate concentration (*Cywinska 2010*).

Intravascular haemolysis is indicated by an increase in free plasma haemoglobin, accompanied by a decrease in serum haptoglobin levels and the presence of haemoglobin pigment in urine. Changes in haptoglobin levels reflect intravascular haemolysis, because haptoglobin binds free plasma haemoglobin, to prevent the latter's excretion in the urine so that the iron and globin can be recycled in the liver. In horses, the serum haptoglobin level has proven to be a useful indicator of infection, inflammation and haemolytic disease (*Cywinska 2010*).

Electrolyte changes

High intensity work can increase heat production 40- to 60-fold over basal metabolism. The major pathway for heat loss in the horse is sweating. According to Al-Qudah et al (2008) a horse can lose up to 40L of fluid during a long distance race, depending on the intensity and duration of the exercise, while Weiss et al (2002) and Reed et al. (2004) found that under hot environmental conditions, fluid losses during an endurance race can reach up to 10 to 15L per hour (*Al-Qudah 2008, Reed 2004, Weiss 2002*). A horse's sweat is hypertonic with respect to plasma, and as a result, large amounts of sodium, chloride and potassium are lost during prolonged exercise (*Flaminio 1998*), along with smaller quantities of magnesium, phosphate and calcium (*Reed 2004, Weiss 2002*). The loss of Na⁺, K⁺ and Cl⁻ together with the fluid, prevents their actual blood concentration from increasing to a level that triggers the thirst response, and can therefore lead to further dehydration because the horse refuses to drink (*Al-Qudah 2008*).

During prolonged exercise sodium and chloride are mainly lost through sweating, while potassium is both lost through sweat and in the gastrointestinal tract, where it is exchanged for sodium (*Reed 2004*). Sweating can also lead to an excessive Ca²⁺ loss, in which case the dehydrated horse can start showing clinical signs of calcium deficiency, such as muscle twitching and spasms (*Al-Qudah 2008*).

The electrolyte losses seem to increase with an increased exercise difficulty level and horses need some time to recover. Ecker et al. (1995) found that there were few changes after a recovery period of 30 minutes and for some horses losses persisted overnight (*Ecker 1995*), but Freeston et al. (1991) found that the maximal exercise-induced increase in plasma potassium levels returned to resting values within 30 minutes post-exercise. They also found that conditioning caused no obvious trends in plasma potassium-recovery (*Freeston 1991*). With regard to body weight and cation content, White (1998) studied horses competing in 3-day, combined-training events. He found a reduction in total body weight and cation content, which was not replenished 12 to 24 hours after the event, even though the serum or plasma concentration of various constituents may have been within normal limits (*White 1998*). Hypochloremia as well as hypocalcaemia, may persist 12 or more hours after speed and endurance tests too (*White 1998*).

In horses that are not well conditioned or in competitions in which terrain, footing, or hot environments increase the thermal load or decrease heat loss, greater losses of fluids and

electrolytes can be expected. Body weight losses exceeding 5% and cation losses exceeding 4000 mEq/L occur in endurance horses suffering from exhaustion and synchronous diaphragmatic flutter. It is likely that the horse's large intestine serves as a reservoir for both fluid and electrolytes to ensure that performance is not diminished (*Reed 2004, White 1998*).

While Ralston et al. (1989) suggested that oral electrolyte supplementation at approximately 20 km intervals during a 96 km race may help to maintain serum concentrations of electrolytes (*Ralston 1989*), Sampieri et al. (2006) found that supplementing endurance horses with high doses of NaCl and KCl did not provide any detectable competitive advantage in 80 km rides other than enhancing water intake (*Sampieri 2006*).

Wijnberg et al. (2002), induced hypocalcemia and hypomagnesemia in Dutch warm blood horses. Although none of the horses showed classical signs of hypocalcemia and hypomagnesemia, all horses had spontaneous activity in the measured muscles indicative of nerve hyperirritability. Calcium and magnesium deficits appear to have consequences, which may be subclinical, affecting functions of the neuromuscular system (*Wijnberg 2002*). During endurance rides, hypocalcemia and hypomagnesemia can be expected, because of losses in the sweat and the binding of calcium to plasma proteins to counteract the metabolic alkalosis due to the chloride losses (*Wijnberg 2002, Weiss 2002*). Weiss et al. (2002) managed to provide their horses with sufficient dietary calcium and magnesium, to prevent any major downward changes in their concentrations, during a 61.7 km ride (*Weiss 2002*).

Lactate

The differences in the rate of muscle lactate production in the horse reflect the degree to which anaerobic metabolism contributes to energy production in each discipline.

Accumulation of lactate in blood is related to the workload; expressed as the velocity at which the horses move during a particular type of exercise (*Desmecht 1996*).

Research by Trilk et al. (2002) demonstrated that a lactate-guided conditioning programme can significantly enhance endurance performance over a 6-week time period when the conditioning protocol is adjusted every 2 weeks based on VLa4 improvement (*Trilk 2002*).

Maximal blood lactate steady state concentration (MLSS) and anaerobic threshold (AT) have been shown to accurately predict long distance event performance and training loads (*Gondim 2007*). In practice, trainers use the 4 mM blood lactate cut-off, a concentration calculated for human athletes, to establish AT and predict training loads for equine athletes. According to Gondim et al (2007), an adapted lactate minimum speed (LMS) protocol for equine athletes seems to be a reliable method for expressing equine endurance athletes' MLSS and the time for lactate to peak. These authors found a minimum speed of 20.75 km/h (\pm 2.06), at which the lactate levels would remain at resting baseline levels during a 10km trail (*Gondim 2007*).

Evans et al. (1995), found that the training induced decrease in blood lactate concentration during submaximal exercise, is not dependent on the intensity of exercise during training (*Evans 1995*).

Materials and methods;

Horses

Two teams of 9 Arab horses were used. Horses in team A had previous experience in endurance competitions of 160 km, while horses in team B had participated in endurance competitions up to 120 km. All the horses were kept under the same circumstances, in a fanned stable with a small sandy paddock attached to it.

Table 1: General information and performance results						
Horse	Age	Gender	Injuries	Nr of SET's completed	Average speed (km/hr)	Competition status
A1	9	Mare	-Traumatic injury left hind limb, soft tissue and lacerations	1	-	-
A2	12	Gelding	-Short gait hind limbs. Stiff gluteal muscles	4	15,3	Completed
A3	11	Gelding	-	4	15,0	Completed
A4	10	Mare	-	3	19,1	Eliminated at 3 rd vet gate → Metabolic
A5	9	Gelding	-	4	17,8	Eliminated at 4 th vet gate → Metabolic
A6	9	Gelding	-Tendinitis	1	-	-
A7	10	Gelding	-	4	17,5	Eliminated at 2 nd vet gate → lameness
A8	14	Gelding	-Left fore limb frog degeneration + thrush	4	12,7	Completed
A9	11	Mare	-	1	13,3	Eliminated at 2 nd vet gate → Lameness
B1	13	Gelding	-Lame grade 3 left forelimb. History of osselets.	2	16,2	Completed
B2	12	Mare	-Mild lameness left hindlimb. Muscle stiffness / myositis. -Lame left forelimb. Volar pouch swelling. History of fetlock injury 1 year ago. -Lame grade 4 left forelimb. -Lame grade 4 left forelimb, severe tendinitis.	2	-	-
B3	12	Gelding	-	4	16,1	Retired at 2 nd vet gate
B4	14	Gelding	-Intra articular bilateral fore fetlocks	2	14,5	Completed
B5	11	Gelding	-Longissimus dorsi spasm -Lame right hindlimb	3	-	-
B6	14	Mare	-Lame right forelimb. Possible mild soft tissue injury.	3	14,9	Completed
B7	12	Mare	-Mild lame left forelimb. history of sesamoid fracture -Mild lame left forelimb. Frog degeneration	2	15,3	Eliminated at 2 nd vet gate → Lameness
B8	13	Mare	-URTI - bilateral purulent nasal discharge	3	13,3	Eliminated at 1 st vet gate → Lameness
B9	11	Mare	Heart Problems	4	-	-

Training regimes

In the month before starting the conditioning regime, none of the horses were trained. During a three months training period the horses were prepared for a 120 km endurance competition. For the first two months, both teams were trained every other day on the same 35 km long track, with one five minute stop exactly in the middle of the route. Using a GPS monitor, the speed was monitored. Team A started out with a speed of 14 km/h and worked up to 20 km/h, while team B started with a speed of 12.5 km/h and worked up to 17.5 km/h. The only criteria used to determine how to increase the speed increased was by monitoring the heart rate of each individual horse with an electrical heart rate monitor. Indications for going faster were a heart rate that did not increase significantly during exercise and/or a heart rate that dropped quickly to pre-ride levels after exercise.

On the days the team of horses was not trained at the 35 km track, they were subjected to strength training. The maximum distance travelled was 20 km and it consisted of a lot of hills. During the third month, the horses were moved to the competition area. Here, the training was increased to 60 km every other day. Because the track conditions, climate and other factors were not exactly the same as those used during the previous SET's, no more standardized exercise tests were conducted in this period.

Standardized exercise test

During the two months training period, standardized exercise tests (SET) were conducted four times. The first SET took place two weeks after the start of the training and the time between a SET per group was approximately two weeks. During the Standardized Exercise Tests (SET), all environmental factors were the same. Both teams were trained at a speed of 15.5 km/h, there was no rain, rider-horse combinations were unchanged and they would start the ride at exactly the same time. For each SET, three blood samples were collected; pre-ride- (taken at the stable, the day before the actual SET), 0 minutes- and 30 minutes post-exercise samples (taken at the end of the track). The heart rate was monitored four times; pre-ride and 0-, 10- and 30 minutes post-exercise, using either a stethoscope or an electrical heart rate monitor. The rectal temperature was monitored three times, i.e. pre-ride, 0- and 30 minutes post-exercise using a digital thermometer. The horses were only fed after the pre-ride sample had been taken.

During each SET, the minimum and maximum values for ambient temperature and relative humidity were measured (table 2 and 3 respectively) and these did not differ significantly.

Table 2: Ambient temperature values

Temp (°C)			
<u>SET</u>	<u>Min</u>	<u>Max</u>	<u>Mean</u>
<u>1</u>	31,1	32,0	31,6
<u>2</u>	27,9	29,7	28,8
<u>3</u>	31,0	33,1	32,1
<u>4</u>	28,7	30,8	29,8

Table 3: Relative humidity values

Rel. Humidity			
<u>SET</u>	<u>Min</u>	<u>Max</u>	<u>Mean</u>
<u>1</u>	65,3	68,7	67,0
<u>2</u>	76,4	82,1	79,3
<u>3</u>	58,6	67,8	63,2
<u>4</u>	72,2	79,4	75,8

Blood samples

Blood samples were put on ice immediately after collection and analysed in the laboratory within a few hours. Using a Hitachi 902 Automatic Biochemical Analyzer; concentrations of lactate, Ca²⁺, Na⁺, K⁺, Cl⁻, phosphate (all in mmol/L) and of the muscle enzymes; aspartate aminotransferase (AST) and creatine kinase (CK) (both in U/L), were determined. Blood neutrophil (B and S), lymphocyte, monocyte, eosinophil and basophil (all in 10⁹/L) concentrations were counted manually. The red blood cell (RBC 10¹²/L), white blood cell (WBC 10¹²/L), haemoglobin (g/L) and thrombocyte (10⁹/L) counts were determined using an Abott Cell-Dyn 3700 machine. The PCV (L/L) was determined manually after spinning heparinized blood in a Hettich centrifuge. The mean cell volume (MCV in f/L) and mean cell haemoglobin concentration (MCHC in g/L) were also calculated manually.

Statistics

The results were analysed statistically using SPSS v 16. A Shapiro-Wilk test was used to check for a normal distribution of the data. After which, a one-way-ANOVA regression analysis was used to analyse normally distributed data, and a Mann-Whitney test for non-normally distributed data. In all cases, differences were considered significant if p < 0.05. A Chi-square test was performed (see table 4) to see if there was a relationship between the number of SET's a horse participated in and the result of the competition.

Results

Table 4; Participation of individual horses

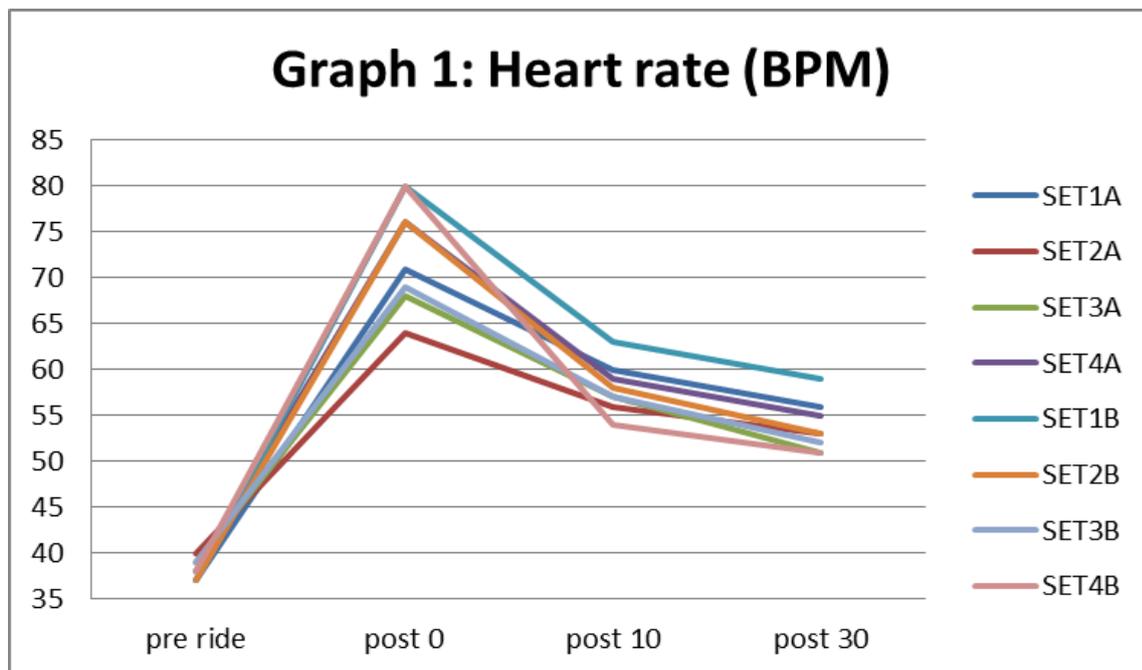
	SET1	SET2	SET3	SET4	Start Comp	Complete Comp
Team A	8 -A9→ ND	6 -A1→ LOC -A6→ LOC -A9→ ND	7 -A1→ LOC -A6→ LOC	5 -A1→ LOC -A4→ ND -A6→ LOC -A9→ ND	7 -A1→LOC -A6→LOC	3 -A4→VG3 -A5→VG4 -A7→VG2 -A9→VG2
Team B	8 -B8→ RES	9	5 -B1→ ND -B2→ LOC -B4→ LOC -B7→ LOC	3 -B1→ ND -B2→ LOC -B4→ LOC -B5→ LOC -B6→ LOC -B7→ LOC	6 -B2→LOC -B5→LOC -B9→DCD	3 -B3→VG2 -B7→VG2 -B8→VG1

00=nr of horses participating, AB0= not participating horse, ND=no data, LOC= locomotory, DCD= deceased, VG= vet gate.

In team A, 4 out of the 9 horses endured some form of locomotory problems, as a result of which 2 horses were withdrawn from the competition. Seven out of the 9 horses from team A started the competition and 3 completed the race successfully. In team B, 6 out of 8 horses endured some form of locomotory problem, as a result of which 2 were withdrawn from the competition. 6 out of the 8 horses from team B started the competition, and 3 completed the race successfully. All of the eliminated horses (total = 7) were eliminated because of locomotory problems, except for horse A4 and A5 which developed metabolic problems due to the very high average speed at which they were racing (19 and 18 km/h respectively).

At an $\alpha = 0.05$ significance level, there was no correlation between the number of SET's a horse participated in and it's result in the competition. Table 4 shows the number of horses participating in each SET and the competition and the general reasons why the individual horses did not participate. More details on the horses and their injuries can be found in table1.

Heart rate



Graph 1 shows the general trend for the heart rate, where the maximum is reached immediately after exercise, and subsequently drops more readily during the first ten minutes after exercise than between the ten to thirty minutes post exercise. At thirty minutes post exercise, the mean heart rate level did not yet return to pre-ride values, but it is below 60 beats per minute.

The heart rate (see table 5) showed no significant differences between team A and Team B for any of the pre-ride samples, which is to be expected with horses kept under the same circumstances. The only time the less experienced team B had a significantly higher heart rate was immediately after finishing the ride (0- minutes post exercise) for the first and second SET's. This suggests that team B, following its own training regime, managed to reach an equal fitness level compared to team A, who had more experience at the start, within one month. There are no significant differences between the teams for the 10- and 30 minutes post-exercise samples, which shows that the difference between the teams at 0 minutes post exercise can be recovered within ten minutes. The results seem to show a trend to a decrease in the mean heart rate with every SET; however, no significant differences could be found.

Table 5: Heart rate of endurance horses trained following different regimes, measured pre-ride and at post 0-, post 10- and post 30 minute intervals during consecutive exercise tests (SET)

Phase	Heart Rate (BPM)							
	Pre Ride		Post 0 min		Post 10 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	37 ± 4 n=7	37 ± 7 n=8	71 ± 6* n=8	80 ± 9* n=7	60 ± 4 n=8	63 ± 9 n=7	56 ± 5 n=8	59 ± 10 n=7
SET 2	40 ± 5 n=6	37 ± 3 n=8	64 ± 7* n=6	76 ± 9* n=8	56 ± 7 n=6	58 ± 4 n=8	53 ± 3 n=6	53 ± 7 n=8
SET 3	39 ± 5 n=6	39 ± 5 n=6	68 ± 4 n=7	69 ± 3 n=4	57 ± 3 n=7	57 ± 3 n=4	51 ± 4 n=7	52 ± 3 n=4
SET 4	38 ± 5 n=5	38 ± 8 n=2	76 ± 8 n=5	80 ± 0 n=2	59 ± 4 n=5	54 ± 8 n=2	55 ± 3 n=5	51 ± 10 n=2

All values are expressed as mean ± standard deviation.

*One way ANOVA $p < 0,05$: SET1post0, SET2post0

Rectal temperature

The rectal temperature (table 6), shows a significant difference between team A and team B for the pre-ride samples of the second and third SET. For the 0-minute post-exercise measurement of the third SET, the mean rectal temperature of team B was significantly lower than that of team A.

Table 6: Rectal temperature of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive exercise tests (SET)

Phase	Rectal Temperature (°C)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	37,6 ± 0,20 n=7	37,5 ± 0,18 n=8	40,2 ± 0,91 n=8	40,6 ± 0,93 n=7	38,7 ± 0,78 n=8	39,0 ± 0,84 n=7
SET 2	36,9 ± 0,32* n=6	37,6 ± 0,10* n=8	39,9 ± 0,42 n=6	39,8 ± 0,85 n=8	38,6 ± 0,17 n=6	39,1 ± 0,60 n=8
SET 3	37,1 ± 0,27* n=6	37,5 ± 0,29* n=6	40,1 ± 0,44* n=7	39,6 ± 0,00* n=4	39,1 ± 0,74 n=7	39,2 ± 0,37 n=4
SET 4	37,2 ± 0,17 n=5	37,4 ± 0,14 n=2	40,2 ± 0,65 n=5	40,5 ± 0,35 n=2	38,9 ± 0,29 n=5	39,6 ± 1,13 n=2

All values are expressed as mean ± standard deviation.

*One way ANOVA $p < 0,05$; SET2pre, SET3pre, SET3 post0

Overall, there were twelve different blood samples, because on each of the four SET's, three samples were taken from each horse. The measurement results of each variable was analysed statistically to see if there were significant differences between team A and team B. The variables that showed significant differences ($p < 0.05$) between team A and team B for three or more samples are shown in tables 7 – 13. These were; aspartate aminotransferase activity (33%), plasma sodium concentration (42%), plasma chloride concentration (33%), lactate concentration (33%), white blood cell count (42%), segmented neutrophils count (50%) and thrombocyte count (50%).

Electrolytes

The electrolytes that show a significant difference between teams A and B for more than 25% of the results, were the plasma sodium- and chloride concentrations.

The mean sodium concentration in plasma (table 7) was significantly higher for team B during the first SET 0 minutes post exercise and the second and third SET pre-ride. From the 0 minutes post exercise onwards, the sodium concentration of team A was higher than team B, but only significantly for the 30 minutes post exercise sample of the third SET and the 0 minutes post exercise of the fourth SET.

Table 7: Plasma sodium concentration of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive SETs

Phase	Sodium (mmol/L)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	139,1 ± 1,36 <i>n</i> =7	138,7 ± 1,25 <i>n</i> =8	137,0 ± 2,06* <i>n</i> =8	140,3 ± 1,83* <i>n</i> =7	138,2 ± 2,44 <i>n</i> =8	140,3 ± 1,57 <i>n</i> =7
SET 2	137,0 ± 1,34* <i>n</i> =6	141,0 ± 1,54* <i>n</i> =8	137,8 ± 1,90 <i>n</i> =6	139,4 ± 2,15 <i>n</i> =8	137,0 ± 1,35 <i>n</i> =6	137,4 ± 1,54 <i>n</i> =8
SET 3	134,8 ± 1,16* <i>n</i> =6	141,5 ± 0,78* <i>n</i> =6	141,2 ± 3,42 <i>n</i> =7	141,0 ± 2,25 <i>n</i> =4	142,0 ± 2,50* <i>n</i> =7	139,0 ± 1,03* <i>n</i> =4
SET 4	Nd	150,4 ± 3,33 <i>n</i> =3	148,4 ± 2,94* <i>n</i> =5	142,1 ± 1,70* <i>n</i> =2	145,8 ± 3,14 <i>n</i> =5	139,8 ± 0,99 <i>n</i> =2

All values are expressed as mean ± standard deviation.

**One way ANOVA $p < 0,05$; SET2pre, SET3pre, SET1post0, SET4post0, SET3post30*

The significant differences between team A and team B for the plasma chloride concentration (table 8) could be found mainly during the pre-ride samples, namely those of the first, second and third SET. The 0 minutes post exercise sample of the third SET differs significantly between the two teams too. For the first three SETs, the mean plasma chloride concentration of team B is consistently higher than team A.

Table 8: Plasma chloride concentration of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive SETs

Phase	Chloride (mmol/L)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	99,6 ± 0,42* n=7	101,4 ± 1,27* n=8	91,5 ± 2,64 n=8	93,2 ± 2,40 n=7	93,6 ± 2,73 n=8	94,5 ± 2,36 n=7
SET 2	98,2 ± 1,04* n=6	100,7 ± 1,39* n=8	93,3 ± 0,98 n=6	93,8 ± 1,18 n=8	93,5 ± 0,80 n=6	93,8 ± 0,92 n=8
SET 3	97,5 ± 1,01* n=6	101,8 ± 1,59* n=6	92,8 ± 2,57* n=7	96,9 ± 2,06* n=4	94,7 ± 0,95 n=7	96,8 ± 2,70 n=4
SET 4	Nd	108,4 ± 3,79 n=3	97,9 ± 2,00 n=5	95,2 ± 1,91 n=2	97,6 ± 3,61 n=5	95,5 ± 2,19 n=2

All values are expressed as mean ± standard deviation.

**One way ANOVA $p < 0,05$; SET1pre, SET2pre, SET3pre, SET3post0*

Both the mean potassium and calcium concentrations only showed a significant difference for the second SET pre-ride samples, where the mean blood levels for team A were higher than those for team B. The phosphate blood levels of team A were significantly higher than team B for the pre-ride samples of the first SET, but other than that there were no significant differences between the two teams.

Muscle enzymes

The muscle enzyme aspartate aminotransferase (AST, table 9) varied significantly between team A and B during SET 2. For this SET, both the pre-ride and 0- minute post-exercise samples showed mean AST activity that was significantly higher for team B.

There were no significant differences found between the creatine kinase (CK) activity of both teams.

Table 9: Aspartate aminotransferase activity of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive SETs

Phase	Aspartate aminotransferase (U/L)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	305,1 ± 49,5 n=7	511,0 ± 205,9 n=8	345,8 ± 50,7 n=8	581,4 ± 353,1 n=7	341,2 ± 55,4 n=8	577,0 ± 349,1 n=7
SET 2	273,4 ± 29,4* n=6	435,3 ± 148,2* n=8	314,7 ± 34,4* n=6	477,8 ± 161,2* n=8	305,3 ± 37,0 n=6	464,7 ± 157,6 n=8
SET 3	322,3 ± 70,9 n=6	383,2 ± 123,3 n=6	354,5 ± 74,1 n=7	403,7 ± 97,0 n=4	353,3 ± 78,5 n=7	389,3 ± 92,7 n=4
SET 4	Nd	351,3 ± 73,4 n=3	478,2 ± 167,3 n=5	346,5 ± 112,4 n=2	459,7 ± 172,0 n=5	339,2 ± 102,6 n=2

All values are expressed as mean ± standard deviation.
**Mann-Whitney test p<0,05; SET2pre, SET2post0*

Lactate

The blood lactate concentration (table 10) showed a significant difference for the pre-ride samples of the second and third SET, where the team B lactate levels are significantly higher than team A. And the third SET 0- and 30- minute post-exercise samples also showed significant differences, but here the team A lactate levels were significantly higher than for team B. When the different SET's were statistically compared to each other, there were significant differences for the pre-ride 0- and 30- minute post-exercise samples.

Table 10: Blood lactate concentration of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive SETs

Phase	Lactate (mmol/L)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	0,86 ± 0,21 n=7	1,07 ± 0,24 n=8	3,78 ± 1,55 n=8	5,01 ± 2,77 n=7	2,95 ± 1,24 n=8	3,58 ± 1,36 n=7
SET 2	1,07 ± 0,35* n=6	1,96 ± 0,19* n=8	2,06 ± 0,54 n=6	2,54 ± 0,97 n=8	1,70 ± 0,35 n=6	1,86 ± 0,59 n=8
SET 3	0,97 ± 0,05* n=6	2,00 ± 0,27* n=6	3,24 ± 0,95* n=7	1,68 ± 0,41* n=4	2,46 ± 0,85* n=7	1,40 ± 0,32* n=4
SET 4	Nd	2,20 ± 0,25 n=3	3,40 ± 1,49 n=5	1,92 ± 0,44 n=2	2,73 ± 1,29 n=5	1,76 ± 0,32 n=2

All values are expressed as mean ± standard deviation.

**One way ANOVA $p < 0,05$; SET2pre, SET3pre, SET3post0, SET3post30*

Blood cells

Table 11: White blood cell count of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive SETs

Phase	White blood cells ($10^9/L$)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	7,75 ± 2,05* n=7	9,73 ± 1,29* n=8	7,73 ± 1,47 n=8	9,40 ± 1,58 n=7	7,75 ± 1,57* n=8	10,4 ± 2,16* n=7
SET 2	8,01 ± 2,46 n=6	8,48 ± 1,71 n=8	7,34 ± 1,84 n=6	9,32 ± 1,73 n=8	6,85 ± 1,69* n=6	9,50 ± 2,01* n=8
SET 3	8,17 ± 2,04 n=6	9,71 ± 1,67 n=6	7,78 ± 1,87* n=7	10,7 ± 1,65* n=4	7,59 ± 1,68* n=7	10,3 ± 1,97* n=4
SET 4	Nd	10,3 ± 1,31 n=3	8,88 ± 1,74 n=5	11,3 ± 1,34 n=2	8,66 ± 1,57 n=5	11,4 ± 0,57 n=2

All values are expressed as mean ± standard deviation.

**One way ANOVA $p < 0,05$; SET1pre, SET3post0, SET1post30, SET2post30, SET3post30*

The white blood cell counts (table 11) were consistently higher for team B. This difference was only significant for the 30 minutes post exercise samples of the first, second and third SET, the pre-ride sample of the first SET and the 0 minutes post exercise sample of the third SET.

The mean segmented neutrophil count (table 12) in the blood was, again, consistently higher for team B, being significantly higher for the first pre-ride sample, the second and third 0 minute post-exercise samples and the first, second and third 30 minute post-exercise samples.

Table 12: Segmented neutrophil count of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive SETs

Phase	Segmented neutrophils ($10^9/L$)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	4,80 ± 1,49* n=7	6,46 ± 0,98* n=8	5,40 ± 1,02 n=8	6,49 ± 1,23 n=7	5,90 ± 1,16* n=8	7,67 ± 1,59* n=7
SET 2	5,03 ± 1,50 n=6	5,46 ± 0,99 n=8	5,20 ± 1,19* n=6	6,95 ± 1,46* n=8	4,93 ± 1,29* n=6	7,33 ± 1,54* n=8
SET 3	5,11 ± 1,29 n=6	5,40 ± 1,68 n=6	5,34 ± 1,50* n=7	7,99 ± 1,12* n=4	5,44 ± 1,38* n=7	7,90 ± 1,74* n=4
SET 4	Nd	6,84 ± 0,44 n=3	6,15 ± 1,61 n=5	8,17 ± 1,22 n=2	6,76 ± 1,39 n=5	8,78 ± 0,44 n=2

All values are expressed as mean ± standard deviation.

**One way ANOVA $p < 0,05$; SET1pre, SET2post0, SET3post0, SET1post30, SET2post30, SET3post30*

Other white blood cell parameters had significant differences for less than 25% of the samples.

The banded neutrophil concentrations in the blood was predominantly higher for team B, but only significantly higher for the pre-ride and 0 minute post-exercise samples of the first SET.

Lymphocyte count was significantly higher for team B for the 30 minute post-exercise samples of the first and fourth SET.

The eosinophil count was significantly higher for team B, only for the 30 minute post-exercise sample of the first SET.

The monocyte and basophil counts did not differ significantly for any of the samples.

The mean thrombocyte concentration (table 13) in the blood was consistently higher for team B than for team A. It was significantly higher for team B, for the pre-ride samples of the first, second and third SET, the 0- minute post-exercise samples of the second and third SET and the 30 minutes post-exercise sample of the second SET. The thrombocyte levels found were all within the reference values boundaries (104-244 10⁹/L)(38).

Table 13: Thrombocyte count of endurance horses trained following different regimes, measured pre-ride and at post 0- and post 30 minute intervals during consecutive SETs

Phase	Thrombocytes (10 ⁹ /L)					
	Pre Ride		Post 0 min		Post 30 min	
	Team A	Team B	Team A	Team B	Team A	Team B
SET 1	138,4 ± 56,4* n=7	199,7 ± 29,4* n=8	167,6 ± 41,9 n=8	195,3 ± 76,4 n=7	157,6 ± 57,6 n=8	185,8 ± 72,7 n=7
SET 2	128,2 ± 56,2* n=6	205,6 ± 35,6* n=8	132,1 ± 36,3* n=6	195,6 ± 37,8* n=8	147,7 ± 28,5* n=6	199,3 ± 41,6* n=8
SET 3	131,0 ± 37,3* n=7	204,5 ± 34,2* n=6	127,3 ± 42,0* n=7	187,8 ± 32,3* n=4	139,8 ± 43,1 n=7	191,0 ± 38,9 n=4
SET 4	Nd	183,3 ± 20,3 n=3	134,2 ± 34,2 n=5	170,5 ± 6,4 n=2	123,0 ± 34,5 n=5	178,0 ± 1,4 n=2

All values are expressed as mean ± standard deviation.

**One way ANOVA $p < 0,05$; SET1pre, SET2pre, SET3pre, SET2post0, SET3post0, SET2post30*

Other blood parameters measured that differed significantly between team A and team B in less than 25% of the samples taken, were the concentrations of red blood cells, haemoglobin and plasma proteins, the packed cell volume, the mean cell volume, the mean cellular haemoglobin concentration and the icterus index.

The red blood cell and haemoglobin concentrations, the packed cell volume and the plasma protein concentration did not differ significantly between the two teams for any of the samples taken. The mean cell volume was only significantly higher for team A during the third SET 30 minute post-exercise samples. The mean cellular haemoglobin only showed a significant difference for the second SET pre-ride (team A higher) and the third SET 0 minute post-exercise (team B higher). The icterus index was significantly higher for team B for the third SET during the 0- and 30 minute post-exercise samples.

Discussion

During this study, some of the horses could not join certain standardised exercise tests due to injury problems (table 1 and table 4), primarily of the locomotory system. During the fourth SET, there were only three horses, one of which (B9) was not included in the results, because the horse had a severe cardiac condition. As a result of this condition, horse B9 died of a heart attack in the third month of training. These problems could have been caused by the training regime, although on most occasions horses were prevented from participating in a SET as a precautionary measure rather because they were really unable to. Furthermore, no correlation could be found between the amount of SET's a horse participated in and the performance at the competition.

Even though team B was a group with older and less performing horses than team A, their performance at the competition seemed to be similar. It is, however, not possible to compare the competition results to the SETs, since the riders, environment and possibly climate varied remarkably. Most of the riders during the competition were foreign and it is despicable if they ever competed with these horses before. Especially since this was a long distance race, a rider can make the difference between a horse completing the race in a good condition or being eliminated.

The number of people participating in the sample taking varied a lot. During some SETs there were more people compared to the number of horses, while during other SETs there was only one person. This means that at times there could have been some variation because of the differences between people and at other times the moment of sample taking varied for the individual horses. What's more, some of the people measuring the horse's heart rate and rectal temperature had no previous experience performing those tasks, and as a result did not insert the thermometer into the rectum properly. This probably influenced the absence of significant differences in the first SET and could also explain why the mean pre-ride rectal temperature of team A is below reference value.

At 30 minutes post-exercise, the mean heart rate was below 60 beats per minute, which is important since this is the maximum heart rate level permitted for horses in an endurance competition after a maximum of thirty minutes rest. Since these horses were not cooled during the first thirty minutes, it can be suggested that the heart rates would have been even lower if cooling had taken place. The results only show that team B has a significantly higher heart rate than team A directly after finishing the ride of the first and second SET. During the third SET their fitness level is not significantly different with regard to the heart rate. However, these results have limited value considering the above mentioned problems regarding the sample taking and small number of horses per group.

The pre-ride samples were taken one day before the SET ride took place and the horses were only fed after the pre-ride sampling. These samples were not taken at the same time of day and could vary from ten 'o clock in the morning till four 'o clock in the afternoon. Since the temperature and relative humidity change during the day, this could explain the fact that some of the results, namely the rectal temperature and electrolytes, show significant differences between team A and B for the pre-ride samples. Since no record was kept of the times at which the pre-ride sampling took place, or the temperature and relative humidity at those times, it is not possible to see if there is a correlation with the results.

The environmental temperature and relative humidity during the actual SET's was measured and minimum, maximum and mean values for each SET are presented in tables 2 and 3. There was no significant difference between the different days.

In general, the electrolyte levels of team B appear to be higher than team A at first, indicating a smaller loss due to sweating. For the later SET's, the team A mean electrolyte blood levels are sometimes higher than team B, which could indicate that as the team B horses improve their overall fitness, their sweating capacity increased to improve heat loss.

The AST levels showed great individual differences, the data did not follow a normal distribution, which made it difficult to detect between-team differences. Looking at the mean AST levels, the team B horses seem to be consistently higher than the team A horses, except for the fourth SET. This could suggest a difference between the two teams despite the lack of significant results. Furthermore, looking at the trend of the mean values, the difference between the two teams does seem to get smaller following each successive SET and, for the last SET, the mean AST value of team A was slightly higher than that of team B. This suggests that team B, which consisted of horses with less competition experience, was able to catch up with team A in less than two months, following these conditioning regimes.

The fact that the lactate levels of the two teams differed significantly for two of the pre-ride samples could be due to the effect of the strength training that was performed that day, or the fact that the samples were taken at different times. Again, team B seemed to catch up with team A regarding fitness level, since the mean team B lactate levels were higher than those of team A up until the third SET pre-ride sample and, from the third SET 0 minute post-exercise sample onwards, the mean team A lactate levels were higher than those of team B. Furthermore, whereas during the first SET the mean lactate level of team B rose up to 5.01 mmol/L, from the second SET onwards it did not exceed the 4 mmol/L barrier. The 4 mM barrier for lactate is used to determine the anaerobic threshold. This is defined as the point during exercise of increasing intensity at which blood lactate begins to accumulate above resting levels, i.e. where lactate clearance is no longer able to keep up with lactate production. (Wilmore 2005) This means that during this training protocol neither team exceeded the anaerobic threshold, except for team B during the first SET at 0 minutes post-exercise.

When comparing the different SET's using the one-way-ANOVA, the Tamhane post-hoc test showed significant differences during the 0 minute post-exercise samples between the second and the fourth SET (the fourth SET levels were higher). Contrary to the expectation that the mean lactate levels would be lower during later SET's, as the condition of the horses improved (as for the heart rate), the sample lactate findings do not support this theory. However, since there were only two horses in team B during the fourth SET, it is very hard to compare this to other SETs.

The significant differences in white blood cell counts between the two teams is mainly explained by the segmented neutrophils. Neutrophil levels can rise quickly to a stimulus (in less than four hours), such as acute stress. The banded neutrophils are a precursor of the segmented neutrophils, so when the banded neutrophil levels are high it suggests that the horses are under so much pressure that they need to use the immature neutrophil reserves from their bone marrow. Team B seems to be under more stress than team A, since their segmented neutrophil levels were consistently higher. This could be explained by the fact that they had less training and experience before the start of the experiment. The banded neutrophil levels were only significantly higher for team B during the first SET, meaning that they were under a greater amount of stress initially, but this did not seem to last for the whole experiment.

Conclusion

Because the horses used in the two teams had a different experience background, no conclusions can be drawn with regard to the efficiency of either one of the training regimes. Although the performance, of horses from both teams, at the eventual 120 km endurance race appeared to be similar, this is difficult to compare to the SET data, since the riders and environment at that particular event were completely different. In this study, it was not possible to determine which parameters had the greatest predictive value regarding the horse's performance, since test groups were small and inconsistent. However, the less experienced team B did show, as expected, significantly higher levels during some of the SETs for heart rate, rectal temperature, aspartate aminotransferase activity, plasma sodium- and chloride concentration, lactate concentration and white blood cell-, segmented neutrophil- and thrombocyte count compared to team A.

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