Evaluation of the ergogenic potential of two feed supplements in endurance horses

Janke van Zyl

BSc (Agric) Animal Science

Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Animal Production Physiology

in the Department of Animal and Wildlife Sciences

University of Pretoria

2014

Supervisor: Professor E.C. Webb

Co-supervisor: Dr. J.A. Oosthuyse

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Declaration

I declare that this dissertation, which I hereby submit in partial fulfilment of the requirements for the degree MSc (Agric) Animal Production Physiology in the Department of Animal and Wildlife Sciences at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Acknowledgements

I would like to thank the following people:

- My supervisor Professor E.C. Webb from the University of Pretoria for his time, encouragement and help.
- Dr J.A. Oosthuyse, my co-supervisor and C.E.O. of HealthTech Laboratories, for funding the study and for his assistance.
- Charmian Willis from HealthTech Laboratories for all her assistance and logistical planning.
- Minesh Mistry, David Gerber, Helia Vorster, Dale Parrish and Tasmin Tyszoxiecki from HealthTech Laboratories for their hard work and many contributions.
- Hadaybit Asalem Endurance stables in Dubai, and in particular Hassan Bin Ali, Harmke Margreta, William Peppin Raj, all the veterinarians, trainers and grooms and other staff who participated in the study.
- Azel Swemmer and Annerita du Plessis-Blount from FDA Laboratories.
- Nicol Schneider from IDEXX Laboratories.
- Professor F. Reyers for his advice and contributions.

Table of contents

Abstract

During prolonged effort, such as endurance exercise, the main sources of energy production for ADP phosphorylation are non-structural carbohydrates and fats. The relative percentage utilization of these two main energy sources varies according to the intensity and duration of exercise. Another important energy source is muscle glycogen. During endurance races muscle glycogen is rapidly depleted. Supplemental concentrate feeding could increase the size of these muscle glycogen stores, which would result in more energy to perform work, and improved performance.

The purpose of this study was to evaluate the potential effects of alternative feed supplements *Schisandra chinesis* and CLA in an attempt to improve the working capacity and performance of endurance horses. A study was conducted on 25 Arabian endurance horses at Hadaybit Asalem Stables in Dubai. These horses were all trained in a similar way for at least 20 days before the study commenced. The horses were randomly divided into four groups, namely CLA group, Control group, Schisandra group and a Combination group. These horses were subjected to an adaptation phase of 10 days during which time no feed supplements were fed (Day 0 - Day 9). Feed supplementation started on Day 10 and continued to Day 19 (10 days).

Basal heart and respiration rates, as well as basal rectal temperatures, were recorded daily. Post exercise heart and respiration rates were recorded on days 0, 5, 10, 15 and 19. Blood samples were collected from the horses on days 0, 11 and 19.

Pooled results indicate that Schisandra had the most pronounced effect on lowering pre-exercise basal heart rate values, as well as lowering post exercise heart rate values. CLA supplementation increased basal respiration rate, decreased blood glucose concentrations by improving glucose uptake from the blood and increased lactate dehydrogenase (LDH) concentrations, resulting in an increased ability of the body to clear lactic acid from the system. The combination treatment group had the highest post exercise respiration rates, most likely due to an increased metabolic rate. Comparisons of treatment effects between day 0 and day 19 suggest that Schisandra had the most prominent effect on lowering respiration rates, both basal and post exercise. CLA's most prominent effect was the opposite, by increasing both basal and post exercise respiration rates. As these two supplements have opposite effects on respiration rate in horses, these two supplements should not be fed together.

Keywords: Adaptogen, endurance, working capacity, additive, LDH.

Chapter 1

Introduction

1.1 Evolution of the horse

The horse, *Equus caballus*, evolved from *Hyracotherium*, also known as *Eohippus* (dawn horse), over a period of 55 million to 10 thousand years ago (Ryke, 1987). About the size of a small dog, eating leaves and fruit, *Hyracotherium* lived in the tropical swamps of North America. *Hyracotherium* spread from North America to Europe and Asia about 25 million years ago (Kaminski & Urbanska, 1979). These distant ancestors had five digits on each foot, which gradually evolved into animals with three toes, known as *Hipparion*. Later, only one digit remained, the hoof, and this animal was known as *Hippidion* (d'Andrade, 1979). As time progressed their limbs became more elongated. Hard hooves and longer limbs enhanced their ability to run at speed. These animals increased in body size and a lengthening of the third metacarpal bone occurred, which resulted in an animal known as *Erybipp* (Kaminsky, 1982). *Erybipp* adapted to life as a fast-moving grazer, with the structure of the teeth and jaw, and the depth of the skull, all modified to suit a grazing lifestyle (Ryke, 1987).

Wild horses of the genus *Equus* evolved on the steppes of central Asia during the Pleistocene epoch, between 1.75 million and 10 thousand years ago (Nobis, 1974). *Equus ferus* was most probably the progenitor of the Arabian breed, which means that the Arabian horse was not originally a desert horse, but a steppe dweller from central Asia. Supporting this assumption is the fact that there is no archaeological evidence of wild horses in the Arabian Peninsula. Various nomadic pastoral groups brought these horses from Central Asia to the Fertile Crescent, the ancient area of fertile soil and important rivers stretching in an arc from the Nile River to Tigris and Euphrates, covering Israel, Lebanon, Jordan, Syria, and Iraq. Just south of the arc is the Arabian Desert. On the east, the Fertile Crescent extends to the Persian Gulf. Geologically, this is where Iranian, African, and Arabian tectonic plates meet. Domesticated horses from central Asia were thus the founding stock for those found in southern Arabia, though horse breeding in Arabia did not appear to flourish until the early middle Ages (Nobis, 1974).

Tribes in Mesopotamia and China domesticated horses over 4500 years ago. Domestic horses were recorded in Greece in 1700 B.C. and in Egypt in 1600 B.C. The Romans standardized horses as a means of sport and recreation. The British organized horse racing and developed the Thoroughbred from the Arabian horses in the early eighteenth century (Erickson & Poole**,** 2004). By breeding 40 royal mares selected for speed from local stocks to three imported Arabian stallions the Thoroughbred breed was born. The Thoroughbred horse has thus been selectively bred only within the last 300 years.

Very few present-day horse breeds can be traced back to pre-historical equine forms. Arguably the most renowned horse breed, the Arabian, is a relatively ancient pure blood breed. Previously called Oriental horses, the Arabian horse is believed to originate from Aryan-type horses and not the Mongolian-type as many believe (de Blomac & Bogros, 1978).

Aryan-type horses were used by the Chamitic people for hunting and fighting. Assyrian warriors rode horses very similar to today's Arabian horses. These horses were also used for plundering expeditions and races during festive days; speed was thus an important trait. As a result of inhospitable environment and strict selection pressure enforced by their owners, today's Arabian horse emerged slowly (Kaminsky & De Andres, 1985).

1.2 Endurance racing

1.2.1 Background and history of endurance racing

In exercise physiology, the word 'endurance' defines the physical and mental capacity to withstand fatigue (Weineck, 1990). Sport competitions can be divided into different endurance categories according to the duration of the physical effort required. Short-term endurance requires effort for 35 seconds to 2 minutes. Medium-term endurance lasts from 2 minutes to 10 minutes. Finally, long-term endurance requires effort for 10 minutes to 6 hours or more (Neumann, 1990).

In 1955, the first modern endurance race, the Tevis cup, was held in California, in the United States of America. A total of 160km was completed in the race which stretched from Lake Tahoe to Auburn (Bergero *et al*., 2005). Today, most one-day endurance rides range from 30 to 160 km. It is not uncommon to encounter races of 200km to be run over two days, or even 500 km to be run over five days (Duren, 2000; Sosa Leon, 1998).

Endurance competitions are extremely difficult from a metabolic point of view, and for this reason, they are subjected to very strict veterinary controls to ensure the horse's health. The races are divided into sections in order to accommodate veterinary inspections, feeding and watering. In between these sections, veterinary inspections take place at the veterinary gates. The horses are also inspected both before and after the race. These inspections serve to ensure the safety and welfare for both horse and rider.

A famous endurance race in South Africa, and also around the world, is the Fauresmith race, held annually in the Free State. The race takes place during winter, with temperatures often dropping below freezing at night, while day time temperatures can be quite high. The race covers a wide range of natural terrain. The extremes of both the temperature and terrain force the horses to adapt to the continual changes brought about by the race. Horses and riders must complete 200km over three days, on average of 66.7 km per day. The horses

stop for a rest and to complete the veterinary inspection at 25km intervals. The first Fauresmith race took place in 1964. This race was organized to settle a debate at the *Landbou Weekblad* magazine, regarding which breed has the best endurance ability, the Arabian or the "Boerperd". The ride was a huge success, with the Arabian breed emerging victorious.

Disaster struck after the 1965 race, when most of the horses appeared to have been poisoned by persons who were opposed to the competition. 97 Horses were entered in that year's race, and sixty of them died. Since 1973, however, the race has been an annual event. In the early years of the race, there were no periodic veterinary inspections to monitor the horses, as is compulsory today.

1.2.2 Veterinary inspections

During the veterinary inspection, factors that are checked include heart rate, respiratory frequency, degree of dehydration and soundness (Burger & Dollinger, 1998). By measuring the heart rate and respiratory frequency during the race, the metabolic activity of the horses can be monitored. During these checks, the heart rate and respiration frequency of the horses have to decrease to below a certain predetermined level before the horses are allowed to continue in the race. This is an indication that the horse has recovered sufficiently from the previous section of the race. If the horse is deemed dehydrated, lame or unfit during the veterinary checks, then the horse and rider will be disqualified from the race.

Pre-race examination

1. A veterinarian is required to check the horse's teeth to ensure that the horse meets the minimum criteria with regards to the age of horses (horses must be at least 5 years old in order to be entered into an endurance race).

2. A veterinarian must assess the horse's habitus and ask the rider about the horse's appetite and water intake.

Habitus is defined as the general attitude of the horse, willingness to trot and interest in the environment, and is graded as follows on the veterinary card:

- "A" – Lively, interested in the environment, trots without much encouragement
- "B" – Slightly depressed, has to be encouraged to trot
- "C" – Depressed, refuses to trot or only trots with major encouragement

3. A veterinarian must assess the horse's metabolic state by examining the following:

Evaluate the heart rate (beats per minute) and quality of the heartbeat through auscultation with a stethoscope, placed on the chest wall over the heart.

Gut sounds, auscultated with a stethoscope.

- "N" - Normal gut sounds
- "↓" – decreased gut sounds
- "↑" – increased gut sounds.

4. A veterinarian must determine the level of hydration by means of a skin fold test, done on the point of the shoulder and measured in seconds (the time it takes for the skin fold to return to normal). The time in seconds is recorded as follows on the veterinary card:

- "1" - ≤ 1 second
- "2" - 1 to 2 seconds
- "3" - 2 to 3 seconds
- "4" - > 3 seconds

5. A veterinarian must assess the mucous membranes, with specific attention to:

The colour and appearance of the mucous membranes under the lip and on the gums (the conjunctiva are only checked if there is reason to be concerned on the horse's health). Grading on the vet card:

- "A" – Normal (pink, moist, glistening)
- "B" – Congested, or dry/sticky
- "C" – Severely congested, dirty red colour

6. A veterinarian must measure the capillary refill time (in seconds). The time in seconds is recorded as follows on the veterinary card:

- "1" - ≤ 1 second
- "2" - 1 to 2 seconds
- "3" - 2 to 3 seconds
- "4" - > 3 seconds

7. A veterinarian must listen to the lungs and measure the respiratory rate. Horses showing signs of respiratory disease may not participate in the ride and shall be referred to the treating veterinarian.

8. A veterinarian must check for lesions and skin lesions on the withers, back, loins, rib cage, chest, shoulders and mouth. Also palpate the back to determine whether it is overly sensitive or painful. Check for lesions on the limbs, hoof conditions and other abnormalities. Grading as follows:

- "A" – No lesions
- "B" – Mild to moderate lesions
- "C" – Severe lesions

9. A veterinarian must evaluate the quality of the horse's shoeing (or hoof care for horses that participate barefoot).

10. A veterinarian must examine the horse for signs of disease and, if indicated, refer the horse to the treating veterinarian.

11. The rectal temperature should be measured by a veterinarian if there is any suspicion of disease. If the rectal temperature is above 38.4°C the horse should be referred to the treating veterinarian for a full clinical examination and, if indicated, treatment.

12.The horse's should be trotted out without a saddle, blanket, bandages or protective equipment, on a loose lead over a distance of forty metres away from, and forty metres back to, the veterinarian to evaluate soundness. If the horse is lame, a vote by a panel of veterinarians shall be called for to determine whether the horse may participate in the ride. Horses should preferably be trotted without being chased.

Grading on the veterinary card:

- "A" – Sound, no signs of unevenness or lameness
- "B" – Uneven, but not consistently lame.
- "C" – Lame

A horse is considered to be lame if it shows a constant deviation or pain during the trot up.

Peculiar and/or abnormal gait which does not warrant elimination shall be recorded on the veterinary card.

13. Muscle tone should also be evaluated by a veterinarian. It is evaluated by palpating the major muscles of the hindquarters, and putting slight pressure on these. Muscles should give under slight pressure. If the muscles appear stiff (not warranting elimination), a note should be made on the veterinary card.

Veterinary inspections during the race

Horses must be inspected at the end of each loop of an endurance race. Endurance rides have mandatory "holds" during the ride where horses must recover sufficiently to meet a specific heart rate parameter ranging from 60 to 68 beats per minute (bpm), depending on race regulations. Once this is achieved, the horses allowed to enter the veterinary gate where they are then checked by qualified veterinarians to ensure the horses are fit to continue. Horses that do not “pulse down” within a specified time, or fail to pass the vet check, are disqualified from the competition. Horses may remain in this hold for a maximum of 20 minutes, after which they must proceed to the veterinary checks. If the heart rate of the horse has recovered sufficiently, the horse may enter the veterinary checks earlier than the allocated 20 minutes.

1. A veterinarian must evaluate the habitus of the horse and ask the rider questions on the horse’s appetite and water intake. Decreased appetite and water intake and behavioural changes during a ride are early warning signs indicating that the horse is not recovering sufficiently.

2. A veterinarian must evaluate the horse’s metabolic state as explained above.

3. The cardiac recovery index (CRI) must be determined by a veterinarian at all rides held under the gate timekeeping rules.

The CRI is used to evaluate the horse’s ability to recover at the time of inspection, and gives a good indication of the horse’s ability to continue with the ride. It is determined as follows:

The horse’s pulse rate is measured. The horse is then trotted over a distance of eighty metres (forty metres away from and forty metres back to the veterinarian), during which the horse’s soundness is evaluated. It takes an average of 28 – 30 seconds for a horse to trot over this distance (depending on the speed at which the horse is trotted). Exactly one minute after the horse started the trot-out (or approximately 30 seconds after completion of the trot-out) the pulse rate is measured again. The CRI is indicated o the veterinary card as 64/60, 48/52 or 44/44, etc.

High CRI’s:

Where the second pulse is higher by between four and eight beats per minute, the veterinarian should assess the relevance based on:

The first pulse rate. The closer the pulse rate was to the maximum allowed heart rate (usually 64 beats per minute), the higher the relevance of a higher second pulse rate.

The horse’s slip time (time taken from arrival at the ride base to presentation to the veterinary panel).

Other parameters evaluated during the inspection of the horse.

Where the second pulse rate is higher by eight or more beats per minute the horse should be called back for a re-examination (approximately fifteen minutes before the horse is to depart on the next loop).

Low CRI's and even CRI's

Second pulse rates lower or equal to the first are an indication that the horse still recovers well and is capable of continuing with the ride.

4. A veterinarian must check the gut sounds. Loss of gut sounds usually is an indication of exhaustion. The reflex/tonus of the anal sphincter should be evaluated when gut sounds are absent. Findings are to be assessed relative to all other parameters that are evaluated.

5. A veterinarian must determine the presence of thumps (when the muscles of the flank contract at the same rate as the heartbeat). The pulse rate of these horses often is below the maximum level allowed. Thumps are an indication of electrolyte imbalance, and a reason for elimination.

6. A veterinarian must auscultate the lungs and determine the respiratory rate.

7. A veterinarian must check for lesions on the withers, back, loins, girth chest, shoulders and mouth, and also palpate the back to determine whether it is overly sensitive or painful. Check for lesions on the limbs (including brushing and overreach marks), hoof conditions and other abnormalities.

8. A veterinarian must check whether all the shoes are still in place if the horse was shod at the start of the race. Lost shoes have to be replaced before the horse is allowed to continue with the ride. The horse must be presented again to the veterinary panel after the shoe was replaced.

9. A veterinarian must check for signs of disease or exhaustion (including dehydration, excessive sweating, muscles tremors or spasms or cramps, glassy eyes, staring, colic, mild abdominal pain, reluctance to move) and, if indicated, refer the horse to the treating veterinarian. Diarrhoea leads to loss of fluids and electrolytes and horses with severe diarrhoea shall be eliminated.

Final veterinary inspections

The final inspection is the inspection done after the horse has completed the last loop of the distance it was entered for. It takes on the same format as the other inspections performed after completion of the other loops, and the horse shall be fit to continue (as if there was another loop).

Release inspections

After completion of a race, all horses must be presented to the veterinary panel for a release inspection before they are allowed to leave the race venue. The veterinarians must evaluate the following criteria:

1. Evaluate the horse's habitus and ask the rider on the horse's appetite and water intake.

2. Evaluate the horse's metabolic state

3. Examine the horse for signs of disease. If indicated, refer the horse to the treating veterinarian for a full clinical examination and, if indicated, treatment.

4. If the veterinarian is of the opinion that the horse is fit to travel he shall complete a release card and hand this to the person responsible.

These strict veterinary control measures at endurances races ensure humane treatment of the horses, and it is in the best interest of the owner, rider and trainers of each horse to ensure that it is fit and healthy at all stages of the race. This lowers the risk of injury as the horse is not pushed beyond its endurance limits.

These inspections ensure that the horses are in good health and are fit to continue in the race. The winning horse is thus the horse that completed the race in the shortest amount of time after passing all the veterinary inspections. Awards are also given to the horse with the best overall condition after completing the race.

1.2.3 Best conditioned horse

The purpose of the award is to determine which of the horses that completed the ride are still in the best condition. Only horses that completed the ride within one hour of the fastest time are considered, with a maximum of ten horses per category.

Points are awarded for four factors:

1. Riding time

Hundred points are awarded to the horse with the fastest riding time. For the remaining horses one point is deducted for each minute or part thereof that the horse's time was slower than the fastest horse. For rides over two days one point is deducted for every two minutes, and for rides over three days one point is deducted for every three minutes.

2. Rider weight

Hundred points are awarded to the heaviest rider that is considered for the award. For other riders one point is deducted for each kilogram weighed in below the weight of the heaviest rider.

3. Average heart rate

The average heart rate for the horse during the ride is calculated. The pre-ride inspection is not considered. An average pulse of forty is used as baseline and is awarded hundred points.

- For an average heart rate between forty-one and forty-five beats per minute, two points per beat above forty are deducted from the allocated hundred points.
- Heart rates between forty-six and fifty beats per minute, ten points plus four points per beat above forty-five are deducted from the allocated hundred points.
- Between fifty-one and fifty-five beats per minute, thirty points plus six points per beat above fifty-five are deducted from the allocated hundred points.
- Between fifty-six and sixty beats per minute, sixty plus eight points per beat above fifty-five are deducted from the allocated hundred points.

4. Veterinary parameters:

Penalty points are calculated as follows, and deducted from fifty:

4.1 Soundness- If the horse was awarded an "A" at the end of the ride, no penalty points are incurred. Horses that were awarded a "B" at the end of the ride are not considered for the award.

4.2 Habitus, mucous membranes, lesions (girth, withers and back) and gut sounds:

- If the horses was awarded an "A" at the end of the ride, no penalty points are incurred.
- If the horse was awarded a "B" at the end of the ride, four penalty points are incurred.
- If the horse was awarded a "C" at the end of the ride, eight penalty points are incurred.

4.3 Hydration (skin fold) and capillary refill time. Both are calculated as follows:

- If the horse ends with a score of "1", no penalty points are awarded.
- If the horse ends with a score of "2", four penalty points are awarded.
- If the horse ends with a score of "3", eight penalty points are awarded.
- Horses that end with a score of "4" are not considered for the award.

The total of all penalty points is subtracted from fifty. If no penalty points were incurred, the horse is awarded fifty points

After calculation of the points for all horses in a category, the best scoring horses are called on to be presented to the veterinary panel. The panel now evaluates the horses primarily on movement; if the horse that was awarded the highest score is still sound in the opinion of the veterinary panel, the best conditioned horse award is made to this horse. If the horse with the highest score is not sound and therefore eliminated from the award, the award will be made to the horse with the second highest score (on condition that this horse is sound) etc.

Points are awarded for the following:

1. Recovery rate (maximum twenty points). This is also known as the "slip time" and is the time between arrival at the check point and presentation to the veterinary panel (entry into the veterinary

area). Slip times are recorded for each gate and an average is calculated at the end of the ride. Allocation of points is based on this average.

2. Cardiac Recovery Index (maximum twenty points). The difference between the first and second pulse rate at each gate is calculated, after which the average for all gates is determined. Allocation of points is based on this average.
3. Final riding time score (maximum thirty points). Points are allocated for the rider's total riding time. The rider with the fastest time is awarded maximum points, and one point is deducted for every two minutes that the other riders went slower than the fastest rider.
4. Rider weight (maximum twenty points). The pre-ride weight of each rider (with tack) is used for this score. The heaviest rider being considered for the award is allocated maximum points, and one point is deducted for each kilogram that the other riders weighed less than the heaviest rider.
5. Lameness score (maximum thirty points). Each horse being considered for the best conditioned horse award is trotted out to allow the veterinary panel to evaluate soundness. If the panel is of the opinion that the horse was sound, maximum points are awarded, and if the panel is of the opinion that the horse was lame, the horse is eliminated from the award (but not from completing the ride).
6. Quality of movement (maximum twenty points). This is evaluated as the horse trots out. Points are awarded separately for two elements (each of which counts ten points):

- Attitude. General appearance, habitus, interest in the surroundings are evaluated.
- Action. Willingness to trot, rhythm of movement and impulsion are evaluated.

7. Metabolic score (maximum ten points). Each horse is evaluated by the same member of the veterinary panel, and points are allocated for skin fold (in seconds), capillary refill time (in seconds), appearance of mucous membranes and quality of gut sounds at the time of this examination. Points are allocated for each of the four parameters, after which an average score is calculated.
8. Lesions (maximum ten points). Points are allocated for lesions (or the absence thereof) in the mouth, on the girth, saddle area and back and on the limbs. Points are allocated for each of the parameters, after which an average score is calculated.

These eight scores are added up, and the award is made to the horse with the highest combined score.

1.2.4 Horse breeds

Various breeds, including Thoroughbred, Quarter Horses, Mustangs, Appaloosas, Morgans, Standardbred, and even Mules, have competed successfully in endurance races (Duren, 2000). However, the breed with the most outstanding performance is the Arabian or Arabian crosses. This is due to their muscle-fibre composition, which has a higher than average percentage of Type I fibers (Bergero *et al.*, 2005). Type I fibres are able to work for extended periods of time under aerobic conditions. These fibres have a slow contraction time and a high resistance to fatigue, resulting in improved endurance performance.

Energy is a measure of a feed's potential to fuel body functions and muscle contraction during exercise. Muscle contraction, in turn, will move the legs and ultimately the horse across the ground during the ride. The endurance horse receives a variety of feed types (fibre, starch, fat, protein) which can be used to fuel muscle contraction. Since horses are not able to eat continuously during a ride, feed must be digested and stored within the body to be used later as fuel during exercise. Stored energy in the form of muscle and liver glycogen, intramuscular and adipose triglycerides along with feed taken in during the ride will provide for muscle contraction. For muscle contraction to occur, the chemically bound energy from feed must be converted into mechanical energy. This conversion process occurs in the muscle cell, and utilises adenosine triphosphate (ATP) for muscle contraction.

There are several factors which will determine both the choice of fuel and the pathway used to generate ATP. These factors include: muscle fibre type, the speed and duration of exercise, type of feed and animal fitness.

Two fundamental reactions regenerate ATP:

1) Aerobic Oxidative Phosphorylation, breaking down carbohydrates, fats and protein, in the presence of oxygen, producing energy (ATP).

2) Anaerobic Glycolysis, breaking down glucose or glycogen into lactic acid.

The horse has three basic types of muscle fibre - Type I, IIA and IIB.

Type I fibres are slow contracting (twitch) fibres while Types IIA and IIB are fast-contracting. Type I and IIA fibres can utilise fuels aerobically while Type IIB fibres have a low aerobic capacity and depend on anaerobic glycolysis for energy generation. It is not surprising that different breeds of horses will have different percentages of muscle fibre types. For example, Quarter Horses typically have more Type IIA and IIB fibres and fewer Type I fibres than an Arabian horses. This would explain why the Arabian is known for endurance.

Table 1.1 Metabolic characteristics of different muscle types

	TYPE I	TYPE IIA	TYPE IIB
Classification	**Slow Twitch**	**Fast Twitch High Oxidative**	**Fast Twitch**
Speed of Contraction	slow	fast	fast
Max. Tension developed	low	high	high
Oxidative capacity	high	intermediate to high	low
Capillary density	high	intermediate	low
Liquid (fat) content	high	intermediate	low
Glycogen content	intermediate	high	high
Fatigability	low	medium	high

From Reece W.O., Respiration in Mammals, in Dukes' Physiology of Domestic Animals, 12th ed., 2004.

While walking, the muscles contract very slowly and expend relatively small amounts of ATP. During this type of exercise, Type I fibres are primarily recruited and energy generation is entirely aerobic. At this speed, the muscle bums predominantly fat. As speed increases from a walk to a trot to a canter, Type I fibres alone are unable to cope. At this point, Type IIA fibres are also recruited. These fibres are also aerobic, but they use a combination of glycogen and fat for energy generation. Glycogen can be metabolised twice as fast as fat, and as speed increases, fat becomes simply too slow to fuel movement. At the canter, Type IIB fibres are recruited. At these speeds, the requirements for ATP has exceeded the ability of the horse to deliver enough oxygen to the muscle to produce the energy by aerobic means. Anaerobic glycolysis takes over as a rapid metabolic pathway and results in lactic acid accumulation. Fatigue soon develops as the pH in the muscle begins to fall.

The speed at which endurance horses typically travel is within the range which can be maintained almost entirely through aerobic energy production. Only during short bouts of sprints and during hill climbing would the energy production shift toward anaerobic means. Therefore, fatigue in an endurance horse is much more likely to result from depletion of glycogen and/or triglyceride stores than lactic acid accumulation.

According to Prince *et al*. (2001) Arabians have superior oxidative capacity in comparison to Thoroughbreds, with the result that Arabians are better adapted to endurance work. These researchers also stated that since Arabians have a smaller frame compared to other horses commonly used for racing, such as Thoroughbreds and Quarter horses, it enables Arabians to travel long distances more efficiently. This is because the cost of locomotion of smaller horses is lower than that of bigger or heavier horses. Furthermore, in comparison to Andalusians, which are used for draft work, Arabians and Arabian crosses have lower lactate concentrations in their muscles after exercise at speeds up to 25km/h (Castejon *et al.*, 1994). This provides further evidence that Arabians are better adapted to long-distance, low intensity work.

1.2.5 The need for supplementation

Endurance horses have very high energy and other nutritional demands. Providing adequate nutrition and incorporating useful supplements into these horses' diets will increase performance. Basic nutrient requirements for horses subjected to high exercise intensities are given in table 1.2 below.

Table 1.2 Basic daily nutrient requirements for horses subjected to high exercise intensities

	Requirements
Energy: DE (Mcal)	34
Protein: CP (g)	1309
Roughage: CF (% of bodyweight)	0.5 – 1%
Calcium (%)	40
Phoshorous (%)	28.5
Vitamin A: I/U	650

DE: digestible energy, Mcal: megacalories, CP: Crude Protein, g: grams CF: Crude Fiber, I/U: International Units. Source: NRC 1989.

Most competitors these days make use of these guidelines for feed formulations. There is still a high demand for additional supplementation over and above the basic nutrient requirements.

Two examples of supplements given to horses in an attempt to improve health and performance are Devils Claw and Echinacea. Devils Claw is said to have anti-inflammatory and pain killing properties (Pearson *et al*., 1999). The active ingredients are various iridoid glycosides, acetylated phenolic glycosides, and terpenoids (Chrubasik *et al*., 2002).

The equine industry typically uses Echinacea as an immune booster to compliment a healthy immune system. Two types of Echinacea are commonly used, namely E. purpurea and E. angustiflora. E. purpurea is more commonly used as it is the cheaper option. Echinacea given as a daily dose of 10-mg/kg of the polysaccharide (active ingredient) over a ten-day period was proven to be an effective immuno-stimulant by Wagner *et al*. (1985). In a study performed by O'Neill *et al*. (2002), Echinacea supplementation was found to increase red blood cell concentration over time.

The purpose of this dissertation is to identify alternative feed supplements, or combinations thereof, which could improve performance in endurance horses without risk of overfeeding the horses with concentrate feeds.

1.3 Hypotheses

1. CLA increases the working capacity and performance in endurance horses.
2. *Schisandra chinensis* increases the working capacity and performance in endurance horses.

3. The combination of CLA and *Schisandra chinensis* increases working capacity and performance in endurance horses above that of supplements when given separately (The two supplements in combination have an additive effect).

Chapter 2

Nutrition and feed supplements for endurance horses

2.1 Brief overview of the equine digestive system

2.1.1 Introduction

The horse, a monogastric herbivore, has evolved to maximize its intake by eating large amounts of roughages such as grass and hay (Bennett, 1980). Since roughages are low in energy content, the horse has to eat large amounts in order to meet requirements. Due to a small stomach size, horses are trickle feeders, grazing up to 16 hours a day.

The digestive tract of a horse consists of two major compartments, namely the stomach and small intestine (precaecal compartment) and the hind gut. Relative to its size, the horse has a very small stomach. The hindgut contains the caecum and the colon (Ruckebusch and Roger, 1988). Roughages (for example hay) eaten by the horse is mainly digested in the hindgut. This is referred to as hindgut fermentation, where microbial fermentation dominates.

As in ruminants, microbial digestion requires a large digestive-fermentation organ where transit of feed can be delayed in order to complete fermentation, especially of cellulose. As opposed to ruminants, horses are low methane gas producers. Food material eaten by the horse can take up to 48 hours to pass through the digestive tract: feed is moved rapidly through the stomach and small intestine, but is delayed in the large intestine. In the stomach, the more digestible feeds (soluble matter) are broken down (Ellis and Hill, 2005). This soluble matter is then digested by mammalian enzymes, producing glucose, amino acids and long chain fatty acids which are absorbed in the small intestine. In the precaecal compartment, glucose is the major end product of carbohydrate digestion. Fermentation of the more fibrous feed and residual starch then occurs in the hindgut, with volatile fatty acids being the main end product of microbial fermentation (Cuddeford, 1999). Other end products of microbial fermentation are methane and heat.

2.1.2 Mouth, lips and teeth

With very sensitive, mobile lips, horses are able to select preferentially (Dulphy *et al.*, 1997), picking only the most nutritious or palatable parts of the available forage. Fibrous plant material requires a lot of mechanical grinding, and therefore there is considerable lateral movement in the jaws of herbivores. The upper jaw is wider than the lower jaw, and mastication occurs only on one side at a time (Rowe *et al.*, 2001).

Horses, like all herbivores, masticate their food well, grinding it down to smaller pieces. This allows for better digestion as increased surface area of the food is exposed to digestive juices throughout the digestive tract.

Chewing also serves to mix the feed and saliva, lubricating the food bolus and allowing smooth passage down the oesophagus. In order for the horse to taste the feed, a small portion of the feed must first dissolve in saliva. Saliva is produced by the three paired salivary glands, namely the parotid, submaxillary and sublingual (Brown, 1987). Chewing of fibrous material stimulates the highest salivary production, and salivary flow in horses can be as high as 50ml per minute. Furthermore, as saliva has a low α-amylase activity, oral starch disappearance is negligible in horses (Radicke *et al*., 1992). Saliva is an effective buffer against acids produced in the stomach and bile secreted continuously by the liver. Saliva is produced only when horses are fed, and it is therefore important to provide horses with a constant supply of forage to ensure maximal saliva production.

Swallowing of food occurs in peristaltic movements: a wave moving from the upper to the lower oesophageal sphincter, moving the food from the mouth to the stomach, whereas, inspiration and tongue contractions facilitate the uptake of fluids into the mouth.

2.1.3 Stomach

At the sight or smell of food, the brain stimulates the endocrine cells in the stomach lining to secrete gastrin. Gastrin signals the stomach to secrete gastric juices. After swallowing, the food arrives in the stomach via the cardiac orifice at the dorsal portion, or fundus. The main functions of the fundus are reception and storage of contents. The stomach contents then move from the fundus to the body, or corpus, of the stomach, where mixing of the food contents with gastric juices occur (Gray, 1992). Next, the food moves to the antrum, which serves as the gastric pump, propelling food past the pyloric sphincter and into the duodenum of the small intestine.

The gastric pH of equines is highly variable, ranging from 1.5 to 7.0, depending on the region of the stomach (Merrit, 1999). As the horse eats, feedstuff tends to layer in the stomach, with coarser material in the upper region of the stomach. Medium density feedstuffs are found in the middle zone of the stomach and high density fluids are found in the lower portion of the stomach (de Fombelle, 2003). The pH in the fundus (near the cardiac sphincter) is usually between 6 and 7. In the middle of the stomach (corpus) the pH is between 4 and 5. The pH is at its lowest in the antrum, which is the glandular part of the stomach. Here hydrochloric acid is secreted by the parietal cells, and the pH drops to 1 – 2 (Meyer *et al*., 1995). The glandular part of the stomach is protected from the acids by a mucous coating. The upper part of the stomach (non glandular squamous portion) is not intended to be exposed to the very acidic stomach acid. Horses which are exercised

often, especially on empty stomachs, or horses that are under stress, tend to tense up their abdominal muscles, forcing the acidic juices to the upper part of the stomach, which does not have a protective mucous coating. The result of this is often gastric ulceration (Lorenzo & Merrit, 2002).

Pasture feeding results in the lowest incidence of ulcers, however ulcers have been diagnosed in foals on pasture. Other factors for example stress and intensity of exercise could also result in ulcers. Feeding more concentrates and less roughage are associated with higher incidence of ulcers, due to a lower stomach pH caused by high starch content of concentrate feeds (Murray, 1989). Endurance horses are often given high concentrations of electrolytes during races (on an empty stomach), which is another major cause for gastric ulcers. Withholding feed is also said to increase the incidence of gastric ulcers.

For gastric emptying to occur, solids must first be ground to a small size and suspended in fluid before they leave the stomach. The distension of the stomach is the primary stimulus to increase gastric motility, however gastric emptying is influenced by meal size as well as meal composition (Meyer *et al.*, 1995). According to Geor *et al.* (2001) addition of corn oil delays gastric emptying. Vomiting in horses is extremely rare, some say impossible, due to the marked tonus of the lower oesophageal sphincter.

The mechanical grinding of the stomach as well as the stomach acids break foodstuff down into smaller pieces. This creates a larger surface area for the various enzymes to further break down and digest the feed. Only protein is digested in the stomach, where stomach acids denaturate proteins and pepsin breaks the protein down to peptides. The majority of enzymatic digestion takes place in the small intestine.

2.1.4 Small intestine

The small intestine performs both digestive and absorptive functions. This is a 3-step process. The first step is the degradation of starch by a group of enzymes called amylase, which is produced by the pancreas. The two major forms of starch, amylose and amylopectin, are broken down into disaccharide and trisaccharide units by the amylase enzymes. The second step involves hydrolysis of the disaccharide and trisaccharide units by the small intestine brush border glycanases (primarily amyloglucosidase, or AMG) to form free glucose units (Gray, 1992). The free glucose units are then transported from the small intestine lumen across the interstitial epithelial cells of the small intestine wall via active transport requiring sodium pumps, as well as facilitated diffusion, into the animal's blood stream (Bird *et al.*, 1996). Facilitated diffusion results from active transport of Na and P by means of Na-P-ATPase across a biological membrane from an area of higher concentration to an area of lower concentration . Since the substances move along the direction of their concentration gradients, energy is not required for this process.

The flow of contents (chyme) through the small intestine is regulated in order to provide mixing of the luminal contents with pancreatic enzymes and bile. This facilitates luminal digestion of carbohydrates, fats and proteins. It also provides maximal exposure of digested nutrients to the mucosa of the small intestine for further digestion and absorption into the bloodstream (Huntington, 1997). Since horses do not have a gall bladder, gall is constantly released from the liver into the small intestine, whether there is food to digest or not.

Peristaltic waves move the chyme through the small intestine at a slow rate. Peristalsis induces mass propulsion by contractions of the longitudinal and circular muscles. Segmentation activity dominates the pattern of small intestine motility, with only short distances traversed by peristalsis during the digestive phase. Segmentation is primarily a result of intermittent circular muscle contractions occurring at different sites on the segment. These contractions induce back and forth mixing, rather than unidirectional propulsion (Merrit, 1999).

Transit is slowed down in the lower small intestine and the ileocecal junction also provides some delay in transit. The contents thus remain longer in the ileum than in the jejunum. Furthermore, precaecal retention time measures the total transit between the mouth and the distal ileum. Retention time of digesta in the foregut is influenced by size of meal as well as meal composition with specific regard to the weight of concentrate to roughage ratio. Gastric emptying is part of the precaecal transit and probably contributes to its regulation. The extent of digestion in the precaecal segment is determined by the enzymatic activity and by the retention time of digesta in the foregut (Meyer *et al*., 1995). Medina *et al*. (2002) found that the mean retention time of the digesta in the precaecal part of the GIT varies from 10% to 20% of the total gastrointestinal mean retention time. This can range between 1.6 hours to almost 10 hours, depending on factors such as feed type and method of feed processing. Retention time of the digesta in the foregut (precaecal compartment) is influenced by meal size as well as the meal composition with regard to roughage to concentrate ratio.

2.1.5 Caecum, large intestine and colon

Before entering the colon, the digesta first enters the caecum. The contents leave the caecum rapidly, but remain in the large intestine for a longer period. The large colon is the main site for microbial digestion in the horse. Here microbial fermentation takes place, and volatile fatty acids, methane and heat are the end products.

Any starch that escapes digestion in the precaecal compartment (stomach and small intestine) will be fermented in the hindgut by microflora. The risk of disrupting the normal function of the caecum and colon is high when large amounts of undigested starch (such as highly fermentable grain) arrive in the hindgut

(Kienzle, 1994). De Fombelle (2003) reports that from 4%, up to 30% of the starch eaten by the horse (in the form of grain) can escape precaecal digestion. According to Brown (1987), starch digestion in the large intestine is almost complete.

The pH of both the caecum and the colon are both slightly acidic to neutral, ranging from 6.4 to 7.0 (Hussein *et al*., 2004). The lowest pH values in both the caecum and the colon, recorded 5 to 6 hours post-feeding, were 6.45 and 6.38 respectively (Hussein *et al*., 2004).

Two types of contractile movements move the digesta through the colon:

1) Stationary haustral contractions (slow segmenting movements in the large intestine that occur every 25 minutes) perform a mixing function and impart high resistance barrier to flow

2) Propulsive movements orientated in an oral to aboral direction.

Antiperistaltic movements also impede digesta flow.

Defecation is a reflex act in which faeces is discharged from the rectum. It can be aided by abdominal pressure produced by muscle contraction with the glottis closed.

2.2 Nutritional management of endurance horses

2.2.1 Brief overview of the nutritional management of endurance horses

According to Harris & Harris (2005), the key to optimal sports performance is the proper production and control of energy. This can be managed by formulating feed that caters to all the requirements of the horse. Arguably the three most important aspects of a diet is the concentration of soluble carbohydrates, supply of fibre to the hindgut, and the time that the feed spends in each part of the digestive system.

The horse evolved as a grazing animal, dependant on an almost constant supply of forage (Bennett, 1980), which is predominantly digested in the hindgut. A horse at maintenance (requiring no extra energy to perform work) will be able to meet its energetic demands from an abundant supply of good quality pastures alone. It is only when humans started using horses for their strength to perform work that supplemental feeding became a necessity.

Originally horses were used for transportation, either by being ridden or by drawing carts, or working in farmlands (animal traction). This resulted in an increased need for energy, as grazing alone could not provide enough energy for these horses to complete their tasks. Moreover, as the horses were used for working during most of the day, they could not eat enough at night in order to compensate for the deficiency. Even if

given sufficient time to graze, this would still not be adequate, as the intake of an average horse is restricted to around 12 kg DM per day (Harris & Harris, 2005). This amount of forage would not have provided the horse with sufficient energy for maintenance, as well as work to be performed for humans. Confinement to stables or small pastures may also limit or even prevent grazing opportunities. The problem that the working or competing horse now faced, is adapting from a constant supply of forages with low energy densities and high fibre contents, to energy dense meals, low in fibre, provided to them twice to three times a day in short feeding bouts (Ellis & Hill, 2005).

Thus, in order to meet the additional demands for energy for work, reproduction, lactation and growth (NRC, 1989), horse owners started offering their horses supplements and concentrates which are energy dense due to a high starch (e.g. oats, barley, wheat and corn), protein (soya, peas or beans) or fat (oil) content. Starch in cereal grains is primarily composed of amylose and amylopectin molecules arranged around a central helium (Julliand *et al*., 2006). In the precaecal compartment (stomach and small intestine), starch is broken down to glucose by the host enzymes. In the fermentation chambers of the hindgut, starch is degraded into volatile fatty acids (VFA) and lactate by microbial activity (Julliand *et al*., 2006).

Horses are very sensitive to changes in their diet, and any change could lead to a number of complications such as impaction, indigestion and colic. Any changes in the diet should be made with care over a period of two weeks or more. The bulk of a horse's diet should be roughage such as hay or pasture. Today, formulating horse feeds is an exact science, based on the exact requirements of the horse, from foals to pregnant or lactating mares, young horses, old horses, ponies, hardworking horses, horses at maintenance as well as feed for specific sport disciplines such as racing and endurance riding.

During prolonged effort, such as endurance exercise, the main sources of energy production for ADP phosphorylation are non-structural carbohydrates and fats (Bergero *et al.*, 2005). The relative percentage utilization of these two main energy sources varies according to the intensity and duration of exercise. Another important energy source is muscle glycogen. During endurance rides, muscle glycogen is rapidly depleted (Snow *et al.*, 1981). Supplemental concentrate feeding could increase the size of these muscle glycogen stores, which would result in more energy to perform work, and improved performance. However, feeding feed with high carbohydrate content to horses could have severe consequences, such as impaction, indigestion and torsion which all results in colic, laminitis, equine rhabdomyolysis (ER) and ulcers. Carbo-loading is not possible in horses as each gram of glycogen stores three grams of water. Feeding feeds high in soluble carbohydrates (used for carbo-loading) would lead to various disorders in horses, such as recurrent exertional rhabdomyolysis (RER) syndrome (discussed under section 2.4.2).

2.2.2 Feeding supplemental fat

Since the competing horse would never be able to meets it energy demands by eating roughage alone, a concentrated feed, such as oats, is given to the horse. But, even if oats were substituted as part of the diet, it would still be unlikely that a very hard working horse could eat enough to replace all energy expended. However, if an energy dense oil was added to the diet, it is possible to match this energy requirement with intake (Kronfeld, 1996), as fat liberates 2.5 times the amount of energy per gram than carbohydrate. This could have a glucose sparing effect in the Krebs cycle (Shephard, 1982).

Feeding energy dense oil has many benefits, as listed below (Harris & Harris, 2005). It:

- Reduces the amount of concentrates that needs to be fed to maintain energy intake
- Retains a healthy fibre intake despite a high-energy requirement, as the horse will still be able to eat fibre (hay) after eating a relatively small, yet energy dense meal.
- Increases the energy density of a feed so that the horse effectively takes in more energy, even if smaller amounts of feed are provided, or if appetite is low.
- Decreases heat load as a result of the more efficient conversion to mechanical energy than is achieved with fibre or carbohydrate. This makes the addition of oil useful under hot and humid conditions.
- Potentially helps reduce bowel ballast and water requirements, as water is released when fats are digested.
- Prevents problems associated with feeding a high starch meal to horses, e.g. equine rhabdomyolysis (ER), gastric ulcers and colic associated digestive prolems.
- Addition of corn oil delays gastric emptying according to Geor *et al.* (2001).

For endurance horses, Duren (2000) suggests a grain diet, top-dressed with 7 to 10% fat, with an upper limit of 20%, which is the threshold for palatability. Many fat sources are used in the diets of endurance horses, the most popular being corn oil and soybean oil because of their favourable omega 3 to omega 6 fatty acid ratio.

The omega-3 family stems from alpha-linolenic acid (ALA), and the omega-6 family originates from linoleic acid (LA). ALA and LA are considered “essential fatty acids” as they cannot be manufactured in the body and must be obtained from dietary sources (Duren, 2000).

Omega-3 and omega-6 fatty acids must be balanced within the body in order for both to be effective. Each fatty acid is necessary for the production and distribution of hormone-like substances called prostaglandins (Spangfors, 2000). The prostaglandins that evolve from consumption of omega-3 and omega-6 fatty acids have different effects on inflammation processes in the body.

The optimal ratio of omega-3 fatty acids to omega-6 fatty acids for horses of various ages and uses has not been determined, however researchers believe a ratio of 2 - 4:1 to be optimal (Spangfors, 2000).

The natural diet of horses (primarily grazing) contains more omega-3 fatty acids than diets consisting of concentrate feeds. Domesticated horses are often fed concentrated sources of energy in the form of grain meals. Concentrate feeds possess more omega-6 fatty acids than forage, creating an inappropriate balance of omega-3 to omega-6 fatty acids, especially when diets are high in grain.

Soybean oil is the best of these oils as it has a better omega 3 to omega 6 ratio which results in better anti-inflammatory ratios (Duren, 2000). Spangfors (2000) recommends feeding a fluid vegetable fat with 30% linoleic acid.

Lipolysis (breakdown of stored fat) yields one glycerol molecule and three non-esterified fatty acids (NEFA) which are released into circulation. Glycerol is used by the liver for hepatic gluconeogenesis (Lucke, 1982), the process of generating glucose from non-glucose precursors. Other sources of NEFA, especially butyrate, is produced in the hindgut during fermentation of the feed. NEFA diffuse across a concentration gradient into muscle where it is used for energy supply (Lucke, 1982).

The metabolism of fatty acids play a significant role in energy supply, as aerobic endurance work in horses requires mostly NEFA as a source for ATP regeneration. Supplementing fats to endurance horse feeds could thus be beneficial in improving performance (Bergero *et al.*, 2005) by increasing exercise duration (Snow *et al.*, 1983).

Long-term oil supplementation has been reported to result in improved performance as a result of several mechanisms:

- Increased mobilisation and improved utilization of free fatty acids (FFA), and increased speed of mobilisation (Harris, 1997).
- Increased speed of uptake of FFA into muscle (Orme *et al.*, 1997). This process is considered to be rate-limiting.
- Reduced production of waste heat (less energy wasted) and lower lactic acid production in fit endurance horses (Kronfeld *et al.*, 1995).

- A glycogen-sparing effect, which delays fatigue and improves performance, especially beneficial in endurance activities (Griewe *et al*., 1989).
- Increased pre-exercise muscle glycogen levels (Hughes *et al*., 1995) result in increased capacity for high-intensity exercise (Eaton *et al*., 1995).

Another benefit of supplementing fat has to do with the fact that fat is a good source of important fat soluble nutrients, for example essential fatty acids and fat soluble vitamins. Of these fat soluble vitamins, Vitamin E is the most important, as it has antioxidant properties (Bergero *et al*., 2005).

2.2.3 Water and electrolytes

According to King (1979), the total body water content occupies between 55 and 75% of body weight in all mammals, including horses. The total body water can be divided into compartments: Extracellular- and intracellular fluid compartments. About one third of the total body water is found in the extracellular fluid compartment, with the intracellular compartment taking up two thirds (Guyton, 1986).

The extracellular fluid compartment includes the plasma volume, interstitial space and the transcellular fluid space. The transcellular fluid space includes the gastrointestinal, lymph, ocular and cerebrospinal fluid spaces (Ruckebusch *et al*., 1991). The largest compartment of the transcellular fluid pool in hind gut fermenters is the gastrointestinal fluid space, as the hind gut of a horse contains about 40l of fluid (Sisson and Grossman, 1953), or about 75% of total gastrointestinal fluid (Argenzio *et al*., 1974). The bulk of water lost (two thirds) comes from the intracellular fluid pool (Denny and Dawson, 1975); thus, the extent to which cells can lose water is of major significance in alkalotic hypo-natremic dehydration.

Table 2.1 Daily requirements of Sodium, Chloride. Potassium and Magnesium for horses exercising at different intensities (grams/day)

Electrolyte	Rest	Light work	Moderate work	Heavy work
Sodium (Na^+)	10	20	50	125
Chloride (Cl^-)	10	25	70	175
Potassium (K^+)	25	30	44	75
Magnesium (Mg^{++})	10	11	14	15 - 19

From Pagan, J., D., 1998. Electrolytes and the performance horse. In: J. D. Pagan (Ed.) Advances in Equine Nutrition. P. 201-204. Nottingham University Press. Nottingham, United Kingdom.

Electrolytes are minerals that dissociate in solution into electrically charged ions. Important examples of electrolytes are Sodium (Na^+), Potassium (K^+), Chloride (Cl^-), Calcium (Ca^{2+}) and Magnesium (Mg^{2+}). Electrolytes are lost with faeces, urine and sweat. Sweat loss, in particular, causes the onset of peripheral fatigue and weakness (Bergero, *et al*., 2005). Extracellular and intracellular fluid differs markedly in electrolyte composition. Extracellular fluid contains sodium (Na^+) as the predominant ion, whereas potassium (K^+) is the predominant ion in intracellular fluid. These ions are in osmotic equilibrium under conditions of normal hydration (Edelman, 1958). Sodium in the extracellular fluid provides the basis for this compartment, and so it is also the main determinant of the extracellular fluid volume (Edelman *et al*., 1958).

Movement of water between the two fluid compartments depends on the respective contents of exchangeable cations, Na^+ in extracellular fluid and K^+ in intracellular fluid, together with the anions associated with these cations; chloride (Cl^-) and bicarbonate (HCO_3^-) (Carlson, 1987).

Cell membranes are relatively impermeable to the passive movement of ions. It has long been known that in order to move ions across cell membranes, an active energy requiring process involving sodium pumps is required (Belevych *et al*., 2002).

The main factors causing exhaustion in sports, especially endurance requiring sports are: (Foreman, 1998)

- Fluid and electrolyte loss,
- Acid-base imbalance and
- Intramuscular glycogen depletion.

Water and electrolytes supply is thus of utter importance for endurance horses, as the sweat loss of these can be very high during an endurance race. According to Pagan (1998), sweat losses of 25l per day is not uncommon for endurance horses. The large intestine is able to absorb large amounts of fluid (Argenzio, 1975), and absorption of these gastrointestinal fluids provides an important reservoir of sodium containing fluid (Argenzio *et al*., 1977).

The endurance horse has an enormous ability to compensate for fluid and electrolyte losses by using equilibrium mechanisms between extracellular and intracellular compartments. Fatigue and exhaustion occurs when these compensatory mechanisms fail as a result of severe water and electrolyte losses, coupled with insufficient replacement (Flaminio and Rush, 1998). Excessive fluid loss together with inadequate water intake results in hypertonic dehydration / alkalotic hypo-natremic dehydration (Orloff and Hutchin, 1972).

Water loss during endurance work is a major concern. Schott *et al*. (1997) found that even after overnight recovery, despite a rapid return of plasma volume and ionic concentrations to near normal values, depletion of body fluids and electrolytes is still apparent after the completion of endurance races.

Water intake of a horse in hot, humid conditions can be as high as 90l a day (300% of normal requirement) (Duren, 2000). Sweat losses usually occurs at 2-5 litres per hour of endurance exercise (at a speed of 2-4m per second), but this could be much higher in hot, humid conditions. Fluid and electrolytes should thus be given to replace the fluids and electrolytes lost via sweat: under normal conditions 2-5 litres of water and electrolytes per hour of endurance exercise should be sufficient (Bergero *et al*., 2005). For a 500kg horse during times of extreme sweating, Frape (1988) recommends providing 5l of water with 30g NaCl, or 60g of mixed electrolyte with 15g sucrose or glucose.

Potassium and chloride, two very important electrolytes to an endurance horse due to their many functions in the body, is lost via sweat. Rose *et al*. (1980) has reported significant decreases in these two minerals during long distance exercise. It is thus of utmost importance to replenish these losses with salts administered orally.

Approximate composition of most commercially available electrolyte solutions:

- 45-55% Chloride
- 20-25% Sodium
- 15-20% Potassium
- 1% Calcium & 0.5% Magnesium
- 5-10% Sugar (Dextrose)

Table 2.2 Table Daily electrolyte requirements (g/day) as a function of sweat loss (l)

Electrolyte	**Sweat loss at rest**	**Sweat loss: 5 litres**	**Sweat loss: 10 litres**	**Sweat loss: 25 litres**	**Sweat loss: 40 litres**
Sodium (Na^+)	10	27	43	93	142
Chloride (Cl^-)	10	41	71	163	254
Potassium (K^+)	25	34	43	70	97
Magnesium (Mg^{++})	10	12	13	19	24

From Pagan, J., D., 1998. Electrolytes and the performance horse. In: J. D. Pagan (Ed.) Advances in Equine Nutrition. P. 201-204. Nottingham University Press. Nottingham, United Kingdom.

For each gram of phosphorous to be absorbed through the intestinal wall into the bloodstream, one gram of calcium is needed. If the calcium concentration in the diet are lower than that of the phosphorous, calcium will be mobilized out of the storage depots in the bones in order to facilitate the absorption of phosphorous.

This should be prevented by providing a diet with a calcium:phosphorous ratio of 2:1. As Lucerne is high in calcium, it is beneficial to provide a small amount to endurance horses. A further benefit of providing endurance horses with Lucerne hay is it contains high levels of protein and calcium, which buffers gastric acids, which would prevent the formation of stomach ulcers.

Another important ion for the endurance horse is magnesium. A deficiency of magnesium will result in muscle problems such as cramping or spasms. Magnesium and calcium use the same uptake transport mechanism, but in competition with each other, calcium is commonly the winner since its absorption is regulated by hormones and Vitamin D (Spangfors, 2000). Since there is a competition between magnesium and calcium for uptake into the body, feeds high in calcium (e.g. Lucerne hay) should be fed only in limited amounts.

2.2.4 Feed processing

Feed processing aims to increase the precaecal digestibility and nutritional value of feed by increasing the availability of starch. Rolling, crushing, grinding, roasting, flaking, extruding, steam rolling, steam crushing and pelleting are all examples of feed processing. Of these, grinding is said to have the most benefits.

Grinding alters the form of grains by breaking down the macrostructure of connected starch granules and also by altering the structure of the individual starch granules (Kienzle *et al*., 1994). By grinding the feed, more surface area becomes available for digestive and microbial enzymes to break down the various constituents of the feed. For instance, cereal grains possess a seed coat which inhibit digestion. Once this seed coat is broken by grinding the feed, more complete digestion of the grain takes place.

According to Medina *et al*. (2002), a further benefit of grinding feedstuff is that the smaller the particle size, the longer the retention time. While it is beneficial to decrease the size of the grain particles, it is important to still retain the optimum particle size for maintaining intestinal health, about 1100 microns.

According to Kinzle *et al*. (1994), crushing and rolling is no more effective in decreasing the particle size than chewing. Brown (1987) reports that whatever the process, starch digestion in the large intestine of a horse is almost complete.

2.3 Lactic acid and fatigue

As the intensity of exercise increases, so the amount of oxygen reaching the muscle decreases. This forces the muscle to enter anaerobic oxidation which produces lactic acid and H^+ ions as end products. Lactic acid

decreases the pH of a muscle due to an accumulation of H^+ in muscle fibres. As H^+ continue to accumulate, the cells' physico-chemical buffer capacity and the ability of the cell to transport H^+ out of the cell will be exceeded (Sewell & Harris, 1992).

The drop in pH eventually interferes with contractile processes of the muscle fibres. The mechanisms regulating ADP removal from the myosin-actin cross-bridge sites is also compromised (Sewell & Harris, 1992). This results in failure to maintain ATP/ADP homeostasis, which eventually leads to the inability to perform work.

According to Newsholm *et al*. (1992), the onset of peripheral fatigue is linked to:

1) Reduction of muscle glycogen

2) Increase of lactic acid concentration in the muscle

3) Decrease of blood glucose levels.

2.4 Metabolic disorders in endurance horses related to overfeeding

2.4.1 Overfeeding of feeds containing high amounts of soluble carbohydrates

All sport horses are fed a high-energy grain-based diet in order to meet their high demand for energy. As horses are ill-equipped to digest feeds with high soluble carbohydrate content, this could result in digestive upsets.

Any grains that are not digested precaecally will enter the hindgut (caecum and large intestine) fermentation phase. Here microbes rapidly ferment the grains, producing lactic acid and reducing the pH of the caecum (Rowe *et al*., 2001). This is called fermentative acidosis. The primary microbes responsible for producing lactic acid are *Streptococcus bovis and Lactobacillus* sp (Rowe *et al*., 1994).

A low caecal pH can lead to acidosis (Garner *et al*., 1975), colic, laminitis (Rowe *et al*., 1994) and diarrhoea (Reeves *et al*., 1996). A low caecal pH will also inhibit the growth and function of cellulytic bacteria, which breaks down fibre. The horse will thus not be able to digest roughage properly. When the caecal pH drops very low, cellulytic bacteria will die, releasing endotoxins which is known to cause laminitis in horses (Bailey *et al*., 2002).

Concentrate overfeeding can also result in abnormal behaviour, such as coprophagy in adult horses, weaving, wind sucking, as well as eating wood shavings (bedding) (Ellis and Hill, 2005). Consequently, Meyer *et al*. (1995) suggests not feeding more than 2g/kg bodyweight grain per meal in order to ensure overall intestinal health. This would mean a 500kg horse should not receive more than 1kg grain per meal.

2.4.2 Consequences of overfeeding concentrate feeds to horses

Although feeding concentrated feeds is often necessary, there are certain risks with regard to feeding high starch-based diets to horses. As the horse evolved as a grazing animal, the capacity of the small intestine to digest feeds with high starch content is limited.

If too much starch is fed to a horse, the digestive system could be overwhelmed. The starch (carbohydrates) would undergo rapid fermentation in the hind gut, resulting in a decreased pH in the caecum and large intestine (Murray *et al*., 1996). This decreased pH facilitated the growth of bacteria in the caecum that thrive in acid environments, such as *Streptococcus bovis* and *Lactobacillus* sp (Goodson *et al*., 1988).

Subsequent lysis of those cellulytic bacteria that cannot survive the low pH, results in the release of endotoxins which leads to damage to the mucosa of the caecum and colon (Bailey *et al*., 2002). These toxic compounds could then be absorbed into the bloodstream, with acidosis (Garner *et al*., 1975), ulcers, colic, laminitis (Rowe *et al*., 1994) and diarrhoea (Reeves *et al*., 1996) often being the end result.

The incidence of ulcers is common in horses undergoing rigorous training. Horses that are exercised often, or horses that are under stress (as a result of high starch inclusion in the diet, overtraining, confinement etc.), tend to tense up their abdominal muscles, forcing the acidic juices to the upper part of the stomach. As the upper part of the equine stomach does not have a mucous coating, it is unprotected from the very acidic juices, and the result of this is often gastric ulceration.

High starch-based diets also increase the risk for recurrent exertional rhabdomyolysis (RER) syndrome, also known as Tying-Up disease, Monday Morning sickness or Azoturia. This intermittently occurring disorder is often encountered in racehorses and causes severe cramping of the muscles, most often after exercise and especially in the hind limbs (Harris, 1991). Tying up usually occurs in early onset exercise, whereas RER is usually late onset. RER is also known as exhaustion syndrome in endurance horses.

Until recently it was believed that recurrent exertional rhabdomyolysis was caused by lactic acidosis, but recent studies confirm that RER is caused by an abnormal intracellular calcium regulation, causing the muscle cell to be hypersensitive to stimulation

(Mlekoday *et al*., 2001; Lentz *et al*., 2002). This abnormality is believed to be induced by exercise, and results in excessive muscle contraction and necrosis (as oxygen and nutrient rich blood is unable to reach the muscle fibres when they are contracted) (Lentz *et al*., 1999). Insufficient blood-flow to the contracted muscle tissue, inflammation from the resulting cell damage, and the release of cell contents all cause severe pain to the affected horse.

Affected horses are often reluctant to move, displaying a shortened gait, have muscle cramps, apparent stiffness, may stand hunched or be unable to rise, and may shift weight from side to side. They also display

excessive sweating, elevated heart and respiratory rates; passing reddish-brown urine (myoglobinuria) and dehydration can also be seen (McGowan *et al.*, 2002). Affected muscles are often hard and painful when palpated.

RER is mostly observed in racing Thoroughbreds, but is also common in other breeds such as Standardbreeds and Arabians (Valberg, 2009). In Thoroughbreds, the incidence rate can be as high as 10%, with recurrences of up to 17% (MacLeay *et al.*, 1999). Factors that predispose a horse to RER are sex (females are more predisposed than males), nervous temperament, rigorous training and a high starch diet (MacLeay *et al.*, 1999). RER episodes increase as fitness increase.

Treatment includes calming the horse, relief from muscle pain, rest, replacement of lost electrolytes and fluids, as well as dietary modifications (McKenzie *et al.*, 2003a). These dietary modifications involves substituting starch with fat in the diet and limiting starch sources to less than 20% of daily digestible energy (DE). Substituting starch with fat would also be beneficial in reducing nervousness, as there is a close relationship between nervousness and starch content in feed. Fat can be included in the feed at levels of up to 20% of energy requirement (McKenzie *et al.*, 2003b).

Another disease which is commonly confused with RER is Polysaccharide Storage Myopathy (PSSM). PSSM is characterized by the excessive accumulation of glycogen and an abnormal polysaccharide in skeletal muscle (Valberg, 2009).

Valberg (2009) states that there are at least two forms of PSSM, namely Tyoe 1 and 2.

Type 1 PSSM is an inherited muscle disease caused by the mutation in the GYS1 gene. This mutation is has been found present in over 20 hundred different breeds such as Quarter Horses, Appaloosas, Warmbloods and Tennessee Walking Horses. Only one parent needs to pass on the genetic mutation for the foal to show signs and symptoms of tying up. The chance of their off spring developing PSSM is greater than 50%. In an attempt to eliminate this painful disease, it is advised that horses diagnosed with PSSM should not be bred.

Type 2 PSSM is yet to still be fully understood. A horse diagnosed with Type 2 PSSM displays similar symptoms to a horse with Type 1 PSSM, but would lack the mutation in the GYS1 gene. This means that Type 2 PSSM is not a genetic disorder. Type 2 PSSM is found primarily in Warmblood breeds.

Carbohydrates that are high in starch, such as corn, wheat, oats, barley, and molasses, appear to exacerbate PSSM. In order to increase the energy content of the diet, without including these above mentioned ingredients, extra calories can be provided in the form of fat. An important part of the management of PSSM horses is daily exercise. This enhances glucose utilization, and improves energy metabolism in skeletal muscle.

2.5 Feed supplements

2.5.1 *Schisandra chinensis*

Schisandra is known as a powerful adaptogen, which fights off disease and stress. It is also a hepatostimulant. Schisandra is available in many forms, such as tinctures, infusions, dried berries, and teas, but the most relevant are seed powder and seed extract. The active ingredient of *Schisandra chinensis* is Schisandrin. Pharmacological studies show that Schisandra seed extract increases physical working capacity (Lupandin *et al*., 1986), which results in improved performance and endurance. It also protects against various stressors for example cold and heat stress.

Studies as early as the 1950's show that Schisandra supplementation augments the utilization of oxygen and improves gas exchange in the lungs and cells of the body (Konstantinov, 1959). This increase in oxygen throughout the body leads to decreased lactic acid production, and results in faster recovery of the respiration frequency after intense physical effort. The clearance of lactic acid from the system by the liver also occurs at a higher rate due to the hepatostimulatory effect of Schisandra.

Administration of one dose of *Schisandra chinensis* in mice increased ATP and phosphorylase activities (Belonosov *et al*., 1958). Along with the above-mentioned facts, this is indirect evidence that Schisandra stimulates tissue respiration and energy provision.

Liver mobilization of stored glycogen is stimulated, which increases the levels of glucose in the blood (Lupandin *et al*., 1986). This indicates that the adaptogen stimulated glycogenolysis and suggests an adrenaline-like effect.

Ahumada *et al*. (2006) found that, during exercise, the increase in heart rate in horses is lower when given Schisandra supplement, compared to those who received placebo treatment. Quicker recovery of respiratory frequency, and improved performance in race horses was observed. In polo horses, plasma lactate concentrations were significantly lower when given Schisandra supplement.

Decreasing the lactic acid concentration in muscles and blood would be very beneficial, as increasing lactic acid levels contribute greatly to the onset of fatigue. According to Hancke *et al*., (1994), *Schisandra chinensis* extract might facilitate the recuperation of the cardiovascular, respiratory and metabolic systems in horses submitted to different kinds of exercise. Furthermore, *Schisandra chinensis* has a normalising effect on gastric acid secretion. It suppresses excessive stomach acid production, which prevents gastric ulcers and also speeds up recovery from gastric ulcers (Lapajev, 1978).

Endurance horses experience high levels of stress caused by intense training, confinement, races, concentrated electrolytes given on an empty stomach and energy-dense rations. These high levels of stress often predisposes endurance horses to gastric ulcers. Prevention of gastric ulcers in endurance horses by

Schisandra chinensis would be very beneficial. In cases where there is a hyposecretion of gastric acids, Schisandra increases these levels, however the mechanism by which Schisandra regulates gastric acid secretion is unknown. Schisandra is also known to be anti-inflammatory, which could be helpful in the healing of injuries. It is unclear how Schisandra exerts it anti-inflammatory effect.

Schisandra is only toxic at very high doses (500 – 1000 mg/kg in mice), which has not proven fatal, and recovery is achieved within 24 hours (Kuznetsova, 1958). It seems to be a very well-tolerated feed supplement, with no proven interactions with other drugs. The withdrawal period of *Schisandra chinensis* is unknown.

2.5.2 Conjugated linoleic acid (CLA)

Conjugated Linoleic Acid (CLA) was discovered in the 1980s as an anti-cancer compund found in ground beef. Conjugated linoleic acid is a generic term used to describe geometric and positional isomers of the 18-carbon fatty acid with two conjugated double bonds. CLA's name derives from linoleic acid due to the structural similarities of these two polyunsaturated fatty acids (Ostrowska *et al*., 1999). In humans, in particular, popular benefits of CLA include its anti-carcinogenic properties, ability to reduce the risk of cardiovascular disease, inflammation fighting properties and body weight management properties.

More than 80% of naturally occurring CLA in products such as beef and milk is in the cis-9, trans-11 formation. CLA is produced through a process called biohydrogenation of linoleic acid to stearic acid by rumen bacteria in ruminants. The other important isomer is the trans-10, cis-12 isomer. Each isomer has its own benefits and activities. Benefits of the cis-9, trans-11 isomer include the following properties: anti-carcinogenicity, anti-atherogenicity and immunostimulation. When supplemented on its own, the trans-10, cis-12 CLA isomer has been shown to increase insulin resistance, but when given with the cis-9, trans-11 isomer, this effect is corrected (Song *et al*., 2004). This suggests that the many physiological effects that are reported for CLA are a result of multiple interactions of the biologically active isomers.

Of interest to our study is the ability of CLA to manage body weight, reducing total body fat and increasing lean muscle mass in several animal species (Cook *et al*., 1998). This effect is mostly due to the trans-10, cis-12 isomer (Chin *et al*., 1994).

Interest in feeding CLA to livestock has increased in the last decade as a result of its potential to improve feed efficiency and to increase protein accretion rate (Ostrowska *et al*., 1999). Dugan *et al*. (1997) found that pigs fed CLA deposited less subcutaneous fat and gained more lean mass than pigs fed sunflower oil.

Multiple mechanisms for CLA's effect on body fat reduction have been suggested, such as increasing the energy expenditure, modulating adipocyte (fat cells) metabolism, modulating adipokines and cytokines and increasing fatty acid beta oxidation.

Reducing total body fat could result in improved endurance and performance, as less energy is wasted on carrying unnecessary weight in the form of fat. More lean muscles available for physical work should improve performance in the endurance horse. It is known that reduced body fat could also improve insulin response and reduce insulin resistance.

In rats fed the trans-10, cis-12 isomer, the concentration of serum glucose was significantly increased, and insulin showed a decreasing trend (Akahoshi *et al*., 2003). CLA is known to decrease insulin resistance and improve glucose uptake and utilization by muscles. This effect of CLA on the body was another important interest for our study. Increased levels of glucose in the blood could improve performance, as this increases the amount of readily available energy.

Reduced concentration of total cholesterol as well as an increased concentration of FFA in plasma were observed in pigs by Lauridsen *et al.* (2005), which indicates that CLA supplementation had an influence on lipid metabolism. Increased plasma FFA of CLA-supplemented pigs may indicate higher mobilisation of fatty acids from the adipose tissues compared to sunflower oil fed pigs. Ostrowska *et al.* (1999) reported that serum free fatty acid levels and triglycerides were elevated in finisher pigs fed CLA, and that this effect may result from the inhibitory action of CLA on lipoprotein lipase (LPL) coupled with stimulation of lipolysis in adipose tissue (Park *et al*., 1997).

Beside the adipocyte lipoprotein lipase (LPL) effect, another mechanism by which CLA influences growth, fatness, and energy expenditure has been described to be via a change in leptin level (Corino *et al*., 2002). Leptin treatment has been shown to cause a dose-dependent decrease in food intake, loss of body weight, loss of fat depots and increased energy metabolism (Levin *et al*. 1996; Pelleymounter *et al*., 1995).
It has previously been shown that CLA depresses the activity and gene expression of hepatic 9-desaturase and the desaturase index in porcine subcutaneous adipose (Smith *et al*., 2002; Martín *et al*., 2007) and hepatic (Bretillon *et al*., 1999) tissues. In vitro studies showed that trans-10, cis-12 isomer is responsible for these effects (Choi *et al*., 2000). Therefore, the change in the fatty acids pattern due to dietary CLA appears to be related to an inhibition of the desaturation of abundant fatty acids such as C16:0 and C18:0. Bretillon *et al*. (1999) observed a reduction in the activity of _6-desaturase in rat liver microsomes after dietary supplementation of CLA.

Previous research has shown a rapid alteration of lipid metabolism induced by CLA feeding over a short period of time in pigs (Wiegand *et al*., 2001; Martín *et al*., 2007). Because CLA stimulates lipid mobilization

in mammals, it has been proposed that it could be applied as a management tool to spare energy (de Veth *et al*., 2004; Griinari & Bauman, 2006). Supplementation with CLA lead to the reduction in body fat in rats (Azain *et al*., 2000), hamsters (De Decker *et al*., 1999), chickens (Szymczyk *et al*., 2001) and various other livestock (Cook *et al*., 1998; Houseknecht *et al*., 1998).
Another benefit of CLA is the reduction in adverse effects of immune stimulation (Cook *et al*., 1993) by products such as Echinacea. Concerns are that long term supplementation with immunostimulants could decrease the body's ability to fight off pathogens on its own (when supplementation is stopped). CLA could be effective in preventing this.

Morales *et al*. (2008) and Corino *et al*. (2009) have shown that CLA enhances immune function in piglets and decreases the negative effects of inflammatory response in piglets. This may have a positive effect on overall health and growth. Further investigation established that CLA inhibited carcinogenesis in several animal models (Banni, *et al*., 2003; Pariza *et al*., 2001).

Patterson *et al*. (2008) reported that overall gut health of pigs was also improved with CLA supplementation. As horses have a similar digestion system to pigs it is possible that CLA supplementation could also improve gut health in equines.

There is no evidence that CLA supplementation will induce adverse effects in healthy subjects, even after long term studies in humans and rats (Gaullier *et al*., 2005; Park *et al*., 2005).

Correct nutritional management could improve performance in sport horses by providing sufficient amounts of high quality nutrients, as well as eliminating any nutritional deficiencies. Providing the correct supplements to horses could also increase performance by:

- making more energy available to the equine athlete to perform work
- increasing oxygen supply to the cells of the body
- reducing the effect of stressful conditions, e.g. intense exercise, travelling and fluctuating environmental temperatures, on the body.

Currently there is very little scientific literature on equine supplementation with CLA and *Schisandra chinensis* available.

In this study, we evaluated the possible benefits of providing endurance horses with CLA and *Schisandra chinenis* feed supplements.

Chapter 3

Materials and methods

A study was conducted on 25 Arabian endurance horses at Hadaybit Asalem Stables in Dubai. The purpose of this study was to evaluate the effect of various supplements on the performance of these endurance horses.

These 25 horses, which consisted of 22 geldings and 3 mares, were all in training for endurance races. The ages ranged between 8 to 16 years old, with mean age 11.45 years. The horses were randomly divided into four groups (Table 3.1), namely:

- CLA group (n=7): Consisting of 6 geldings and 1 mare, received 57.92g of CLA containing equal amounts of cis-9, trans-11 and trans-10, cis-12 isomers, mixed with 7.09g maltodextrin (total volume 65g). Average age of horses in this group was 13 years.
- Control group (n=6): The control group, consisting of 5 geldings and 1 mare. Supplement contained no active ingredients, only maltodextrin powder was fed as a placebo (total volume 65g). Average age of horses in this group was 11 years.
- Schisandra group (n=6): Consisting of 6 geldings, received 1.44g Schisandrin daily, mixed with 63.56g maltodextrin (total volume 65g). Average age of horses in this group was 11.5 years.
- Combination group (n=6): Consisting of 5 geldings and 1 mare, received 1.44g Schisandrin and 57.92g CLA mixed with 5.65g maltodextrin (total volume 65g). Average age of horses in this group was 10.3 years.

Table 3.1 Schematic representation of experimental design

Group	Horse number	Age	Gender
CLA	500	10	Gelding
CLA	900	11	Gelding
CLA	1200	15	Gelding
CLA	1400	15	Mare
CLA	1800	14	Gelding
CLA	2100	16	Gelding
CLA	2400	10	Gelding

Average age:		13	
Control	300	9	Mare
Control	1100	8	Gelding
Control	1300	11	Gelding
Control	1600	9	Gelding
Control	1700	12	Gelding
Control	2600	11	Gelding
Average age:		11	
Schisandra	100	13	Gelding
Schisandra	600	9	Gelding
Schisandra	700	12	Gelding
Schisandra	1500	10	Gelding
Schisandra	2000	12	Gelding
Schisandra	2200	13	Gelding
Average age		11.5	
Combination	400	9	Gelding
Combination	1900	8	Gelding
Combination	2300	11	Gelding
Combination	2500	11	Gelding
Combination	2700	10	Gelding
Combination	2800	13	Mare
Average age		10.3	
Overall average age		11.45	

The *Schisandra chinensis*, CLA, Schisandra-CLA mixture and placebo were mixed into the morning feed (500g) of the horses. Total daily intake of concentrated feed was 1.5 kg for all the horses included in the study (500g was fed at 5am, 500g fed at 10:30am and 500g fed at 5pm). All the horses were of similar body weight (±450kg) and body condition and received the same feed ration (see table 3.4 below). The horses ingested all their feed during the study. No additional supplements were fed to these horses for a period 20 days prior to the study, and only the trial supplements were given to the horses during the study. All the horses included in the study were not raced for 20 days before start of the study, and were exercised at medium intensity during these 20 days in order to retain fitness levels.

All the horses were exercised on a treadmill each morning for half an hour (including warm-up and cool down) at an average speed of 1.8m/s. As the treatment groups consisted of only 6 or 7 horses per group, it was decided to exercise these horses at a lower intensity in order to prevent any injury, which would further reduce the number of horses per treatment group. This intensity of exercise did not challenge the horses' metabolism severely, however the veterinarians as well as the researchers feels that this exercise intensity did provide a reasonable indication without adversely affecting the health and wellbeing of these valuable endurance horses. The treadmills were located in an indoor facility with fans used for temperature control. As the study progressed, ambient temperatures steadily increased.

As the study was conducted at the end of the racing season, all the horses were fit and in good racing condition. Water and timothy hay was provided on an *ad libitum* basis. All the horses were housed separately in box stalls, thus we were able to monitor feed and water intake closely.

These horses were subjected to an adaptation phase of 10 days where they did not receive any supplements (Day 0 - Day 9). This period ensured that all the horses received exactly the same treatment, and that the results obtained are comparable between all the horses. Supplementation started on Day 10 and continued to Day 19 (10 days). The study was a double blind study, thus none of the veterinarians, trainers or grooms knew which treatment was given to which horses. Supplement packages were marked A, B, C and D by the laboratory that produced them. Only after completion of the study did the researchers and veterinarians learn the composition of each package.

Three different supplements were fed to the horses in order to evaluate the ergogenic potential of each of these supplements. The duration of the study was 20 days. The study was divided into two stages, namely the adaptation phase and the treatment phase. The adaptation phase is illustrated in Table 3.2 and the treatment phase is illustrated in Table 3.3.

Table 3.2 Schematic representation of the adaptation phase

	CLA group	Control group	Schisandra group	Combination group
Duration	Day 0 - 9	Day 0 - 9	Day 0 - 9	Day 0 - 9
Supplements fed	No supplements fed	No supplements fed	No supplements fed	No supplements fed

Table 3.3 Schematic representation of the treatment phase

	CLA group	Control group	Schisandra group	Combination group
Duration	Day 10 - 19	Day 10 - 19	Day 10 - 19	Day 10 - 19
Supplements fed	57.92g CLA & 7.09g maltodextrin	65g Maltodextrin	1.44g Schisandrin & 63.56g maltodextrin	1.44g Schisandrin & 57.92g CLA & 5.65g maltodextrin

Table 3.4 Analysis of nutrient content given to all the horses included in the study

	Amount measured (DM basis)
CP (g/100g)	11.12
CF (g/100g)	9.73
EE (g/100g)	7.56
Ca (g/100g)	1.03
P (g/100g)	0.52
Mg (g/100g)	0.39
Fe (mg/kg)	325.75
Mn (mg/kg)	105.03
Zn (mg/kg)	188.5
K (g/100g)	0.76
Na (g/100g)	0.50
Cl (g/100g)	1.06

CP: Crude protein, CF: Crude fiber, EE: Ether Extract, Ca: Calcium, P: Phosphorous, Mg: Magnesium, Fe: Iron, Mn: Manganese, Zn: Zinc, K: Potassium, Na: Sodium, Cl: Chloride, DM: Dry Matter. Analysis done by NutriLab, University of Pretoria.

Table 3.5 Analysis of Timothy hay fed to horses included in the study

	DE Mcal/lb	CP %	Ca %	P %
Timothy hay	1.04	11.4	0.36	0.26

DE: digestible energy, Mcal:megacalories, CP: Crude Protein, Ca: Calcium, P: Phosphorous. Source: NRC 1989.

Basal heart and respiration rates as well as rectal temperature of each horse were recorded 1 hour after their morning meal by veterinarians.

Blood samples were collected at the start of the experiment (Day 0), which indicates normal blood parameters. On Day 11 of the trial period, blood samples were collected to monitor the effect of the supplements on the horses after only two doses. On Day 19, blood samples were collected in order to evaluate the long-term supplementation effect of these supplements. Blood samples were obtained at the same time each day: directly after the horses completed their morning feed by the veterinarians. As most horses get very excited prior to feeding time, it was decided to collect the blood samples directly after the horses' morning feed in order to allow them to calm down and for their blood parameters to return to normal.

In order to test the effectiveness of these supplements, the following parameters were evaluated from the jugular blood samples:

1. Haematocrit (erythrocyte count, leukocyte count, haemoglobin concentration) Hadaybit Laboratory (UAE)
2. Differential cell count (neutrophil, lymphocyte, monocyte, eosinophil and basophil %) Hadaybit Laboratory (UAE)
3. Blood pH Hadaybit Laboratory (UAE)
4. Glucose Hadaybit Laboratory (UAE)
5. Lactate Hadaybit Laboratory (UAE)
6. Lactate dehydrogenase (LDH) Hadaybit Laboratory (UAE)
7. Creatinine Hadaybit Laboratory (UAE)
8. Lipogram (Cholesterol, HDL and LDL) Hadaybit Laboratory (UAE)
9. Blood Urea Nitrogen (BUN) Hadaybit Laboratory (UAE)

10. Cortisol — Idexx Laboratory (RSA)
11. Insulin — Idexx Laboratory (RSA)
12. T3 (Triiodothyronine) — Idexx Laboratory (RSA)
13. T4 (Thyroxin) — Idexx Laboratory (RSA)
14. Schizandrin plasma and urine levels — FDA Laboratory (RSA)

As these parameters were evaluated at different Laboratories, the following samples were collected (per horse):

Day 0 & Day 11:

- 1 x whole blood and 1 x plasma for Hadaybit Laboratory (UAE)
- 3 x serum for Idexx Laboratory (RSA)

Day 19:

- 1 x whole blood and 1 x plasma for Hadaybit Laboratory (UAE)
- 3 x serum for Idexx Laboratory (RSA)
- 1 x urine sample for FDA Laboratory (RSA)
- 1 x plasma sample for FDA Laboratory (RSA)

The purpose of blood collection was to monitor the horses' normal blood metabolites and the changes over time with the supplementation.

Haematocrit, blood pH, differential cell count and BUN were measured in order to determine the health and metabolic status of the horses. Day 0 is an indication of baseline values for measured parameters. During and after the trial it was possible to determine whether either *Schisandra chinensis* or CLA altered these values. The blood samples collected on day 11 indicated the effect of the supplements after only 2 doses. The blood samples collected on day 19 indicated the effect of the supplements after 10 days of supplementation.

In the horse the spleen acts as a reservoir for erythrocytes, and at rest one third to a half of erythrocytes are stored in the spleen. Racing breeds have a larger than average spleen. Blood cells stored in the spleen can be mobilized into the blood when there is an increased demand for oxygen in the body (e.g. during intense exercise). The release of stored erythrocytes from the spleen into systemic circulation is under the influence of the sympathetic nervous system and circulating catecholamines (Reece, 2004). The smooth muscle wall

of the spleen is innervated by postganglionic sympathetic neurons. Factors that increase sympathetic nervous activity and/or plasma catecholamines like asphyxia, haemorrhage, exercise and excitement will result in splenic contractions. This contraction will increase the number of circulating erythrocytes. As the blood volume remains constant (or decreases slightly), this results in an increase in packed cell volume (PCV), haemoglobin and red blood cell count. Exercise can also cause a reduction in plasma volume, presumably because of a fluid shift from the intravascular to the extravascular compartment as a result of fluid loss through sweating.

The lipogram indicate the amount of fat (in the form of total cholesterol) circulating in the blood, and the components of cholesterol, HDL and LDL. These metabolites were important in evaluating whether the dietary supplements altered the normal baseline values. The major effects of endurance training are increased use of fat with an associated sparing of muscle glycogen, reduced blood lactate accumulation and an increased work capacity (Reece, 2004).

As CLA is said to increase glucogenic substrates, the following compounds were measured in order to determine to what extent it does so: Glucose and Insulin.

Schisandra chinensis could decrease the lactic acid concentration in blood by increasing the rate of lactic acid clearance from the blood by the liver, as well as increasing oxygen uptake and exchange in the body. The increase of lactic acid clearance from the liver would result in higher concentrations of lactate dehydrogenase, the enzyme responsible for the breakdown of lactic acid.

As cortisol is an indicator of long term stress, the concentration of cortisol in the blood indicates whether dietary CLA and/or *Schisandra chinensis* had any effect on reducing stress.

T3 and T4 indicate the metabolic rate of the horse. By measuring these levels, we were able to determine whether these two dietary supplements had an effect on metabolic rate.

Blood and urine collection Procedure

Blood was collected in the different tubes required for each laboratory; EDTA (whole blood, purple cap) and Lithium Heparin (plasma, green cap) for Hadaybit Laboratory (UAE), Serum for Idexx Laboratory (RSA) (yellow cap) and plasma (red cap) for FDA Laboratory (RSA). Samples were inverted 5 times in a gentle manner so as not to damage the sample, and allowed to stand for 1 hour at room temperature (18 degrees Celsius) to allow clotting.

The samples were sent to Hadaybit Laboratory (UAE) for further processing. The EDTA and Lithium heparin samples were analysed on the same day by Hadaybit Laboratory (UAE).

The serum and plasma samples (going to IDEXX (RSA) and FDA Laboratory (RSA) respectively) were centrifuged at 1000 to 1300 RFC (g) for 10 minutes in a swing bucket centrifuge. The serum or plasma was drawn off, and placed in cryotubes, after which these samples were cooled down to below minus twenty degrees Celsius, and shipped to the respective laboratories in RSA (IDEXX or FDA). The samples were kept below minus twenty degrees Celsius throughout. Dry ice was used to transport the samples from Dubai to South Africa, and the cold chain was never broken.

Urine was collected from horses, which are trained to urinate on command, and no catheters were used. In total, 23 urine samples were collected. The urine of the horses was tested by FDA Laboratory (RSA) in order to determine the concentration of Schisandrin.

Horse number 2500 (gelding, 11 years old, group D) was removed from the study on day 16 due to bilateral epistaxis. It is not clear what caused this reaction, and since none of the other horses showed any adverse effects to the dietary supplement, it is believed that this reaction was unrelated to the supplementation.

This study was cleared by the ethics committee of the University of Pretoria, reference number EC061-11.

Statistical analysis

The study was a completely randomized control study, with 25 horses included in the study. The horses were evaluated based on heart rates, respiration rates, rectal temperature, haematological values, blood metabolites and other specific hormone concentrations.

The Control, Schisandra and Combination groups each consisted of 6 horses, whereas the CLA group consisted of 7 horses.

Data were collected from these horses over a trial period of 20 days. Basal heart and respiration rates, as well as basal rectal temperature, were recorded daily. Post exercise heart and respiration rates were collected on days 0, 5, 10, 15 and 19. Blood was collected from the horses on days 0, 11 and 19.

Factors included were:

- Treatment
- Gender
- Days on treatment

The following statistical results were obtained

Pooled results which included the data of the four treatment groups over the entire trial period (this included adaptation phase as well as supplementation period)

Treatment phase which included the data of the four treatment groups from day 10 to day 19 in order to obtain results for the supplementation period only.

Results on specific days, consisting of the data of the four treatment groups on three specific days (day 0, 11 and 19). This was done in order to evaluate the effect of the supplements on the horses for day 0 (no supplements received), day 11 (two doses of supplements) and day 19 (after 10 days of supplementation).

Mean end values (day 19) were compared to the mean baseline values (day 0) for each group in order to evaluate the effect of long term supplementation within each specific group.

LSMeans (least square means) ± SD (standard deviation) were compared by means of multifactorial ANOVA, and significant differences were tested at $P < 0.05$. LSMeans were compared by means of Bonferroni multiple range test.

Chapter 4

Results and discussion

Trial results are presented in the following order:

4.1 Summary statistics

These are the average values (LSMeans ± SD) of the various parameters for all horses over the entire trial period. The values obtained were compared to reference values (when available) for the various parameters measured in this study.

4.2 Pooled results

The results are average values of each treatment group (LSMeans ± SD) for the various parameters over the entire trial period (adaptation phase and treatment phase).

4.3 Treatment phase results

These results are average values of each treatment group for the various parameters during the treatment phase (day 10 to day 19) only. During the trial period the horses were supplemented with one of the treatments.

4.4 Results for specific days

Average values of each treatment group for the various parameters were recorded on specific days during the adaptation and treatment phases in order to evaluate the effect of the various supplements on that specific day, namely days 0, 5, 10, 11, 15 and 20.

4.5 Comparisons between initial (Day 0) and end (Day 19) physiological parameters in each group

Mean end values (day 19) were compared to the mean baseline values (day 0) for each group in order to evaluate the effect of long term supplementation within each specific group.

4.1. Summary statistics of pooled data from Day 0 to Day 19

4.1.1 Pre-exercise basal physiological parameters

Average basal heart rate (beats per minute/bpm) of all horses over the entire trial period (29.9 ± 5.02 bpm) was on the lower end of the reference ranges reported by Huntington *et al*. (2004), Detweiler and Erickson (2004) and Evans (1994).

Mean basal respiration rate (inhalations per minute/ipm) of all horses in the study, over the entire trial period (11.8 ± 3.23 ipm), was also at the lower end of the reference ranges reported by Huntington *et al*. (2004) and Reece (2004). Low basal heart rate and respiration rate indicate that the fitness level of the horses used during this study was high.

Mean basal rectal temperature of all the horses included in this study (36.6 ± 0.59 °C) was slightly below the reference ranges reported by Huntington *et al*. (2004) and Robertshaw (2004). This could be due to cool weather as the trial took place during the winter months, and as the temperature recordings were done early in the mornings (around 5am).

Summary statistics of basal heart rates, respiration rates and rectal temperature for horses included in this study are provided in Table 4.1.1.

Table 4.1.1 Summary statistics of basal physiological parameters (heart rate, respiration rate and rectal temperature) for endurance horses compared to reference ranges

Reference range	LSMean ± SD	Reference range	Reference range	Reference range
Basal HR	29.9 ± 5.02	30 – 40 *(Huntington et al., 2004)*	28 - 40 *(Detweiler & Erickson, 2004)*	25 – 40 *(Evans, 1994)*
Basal Resp	11.8 ± 3.23	8 – 20 *(Huntington et al., 2004)*	10 – 14 *(Reece, 2004)*	
Basal Rectal Temp	36.6 ± 0.59	37.0 – 38.0 *(Huntington et al., 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*	

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).*

4.1.2 Post exercise physiological parameters

These measurements were recorded in order to evaluate the return to normal (resting) heart rate and respiration rates post exercise. As the increase in both heart and respiration rates were low, it was concluded that all horses used in the study were fit and adapted to this intensity of exercise. It is also apparent that the

recovery rate post exercise was fast, as the heart rates neared basal heart rate values at 30 minutes post exercise. The respiration rate remained somewhat higher than the basal respiration rates, but this can be expected, as any excitement or stress causes an increased respiration rate (basal values were obtained after morning feed when horses are at their calmest).

Summary statistics of various post exercise parameters for horses included in this study are provided in Table 4.1.2.

Table 4.1.2 Summary statistics of various post exercise parameters for endurance horses

	LSMean ± SD
HR 0min	33.2 ± 4.32
HR 10min	30.7 ± 4.21
HR 20min	30.2 ± 4.50
Resp 0min	25.4 ± 8.16
Resp 10min	21.6 ± 7.91
Resp 20min	20.8 ± 7.13

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm) 10 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. **LSMean:** least square mean.

4.1.3 Basal haematological values

Mean blood pH value (7.53 ± 0.055) for all horses in the study was well within the reference range provided by Hadaybit Laboratory (UAE) (UAE); however, it is slightly above the reference range according to Davies (2009).

Mean erythrocyte count (6.70 ± 0.606 x 10^{12}g/l) was at the lower end of the reference range provided by Hadaybit Laboratory (UAE) (UAE) and Latimer *et al*. (2003); however, it was slightly lower than the reference range provided by Reece (2004).

Mean haemoglobin value (11.73 ± 0.961 g/dl) was at the lower end of the reference range provided by Hadaybit Laboratory (UAE), Latimer *et al.* (2003) and Reece (2004).

Mean leukocyte count (6.20 ± 1.239 x 10^{9}/l) was at the lower end of the reference range provided by Hadaybit Laboratory (UAE) and Latimer *et al*. (2003); however, it was lower than the reference range provided by Reece (2004).

Average neutrophil percentage (56.4 ± 5.750 %) for all horses was well within the reference range supplied by Hadaybit Laboratory (UAE), Latimer *et al.* (2003) and Reece (2004).

Average lymphocyte percentage (36.64 ± 6.237 %) for all horses was well within the reference range supplied by Hadaybit Laboratory (UAE), Latimer *et al.* (2003) and Reece (2004).

Average monocyte percentage (2.63 ± 1.330 %) for all horses at the lower end of the reference range supplied by Hadaybit Laboratory (UAE) and Latimer *et al.* (2003); however, it was slightly below the reference range as indicated by Reece (2004) . Inflammatory reactions (especially in the gut and respiratory system) increase monocyte numbers, and since the average monocyte numbers of all the horses were at the lower end of the reference range, it was concluded that the horses had no severe inflammatory reactions.

Average eosinophil percentage (1.22 ± 0.654 %) for all horses was at the lower end of the reference range supplied by Hadaybit Laboratory (UAE) and Latimer *et al.* (2003); however, it was slightly below the reference range according to Reece (2004). Because eosinophil numbers increase during allergic reactions, it can be concluded that none of the horses had severe (or any) allergic reactions to the supplements during the trial.

Average basophil percentage (0.11 ± 0.538 %) for all horses was at the lower end of the reference range supplied by Hadaybit Laboratory (UAE), Latimer *et al.* (2003) and Reece (2004). Basophil numbers were at the lower end of the reference range (close to 0), but as it is normal for horses to have no basophils in their blood, this is of no concern. Basophil numbers increase during allergic reactions and inflammatory processes. A very low basophil count is thus desired. Summary statistics of haematological values for horses included in this study are summarized in Table 4.1.3.

Table 4.1.3 Summary statistics of haematological values for endurance horses compared to reference range

	LSMean ± SD	Reference range	Reference range	Reference range
Blood pH	7.53 ± 0.055	7.5 – 8.5 *(Hadaybit Laboratory (UAE))*		7.35 – 7.45 *(Davies, 2009)*
Ery	6.70 ± 0.606	4.8 – 13 *(Hadaybit Laboratory (UAE))*	6.0 – 10.4 *(Latimer et al., 2003)*	7 – 11 *(Reece, 2004)*
Hgb	11.73 ± 0.961	9 – 15 *(Hadaybit Laboratory (UAE))*	10 – 16 *(Latimer et al., 2003)*	11.5 – 16 *(Reece, 2004)*
Leu	6.20 ± 1.239	5 – 11 *(Hadaybit Laboratory (UAE))*	5.6 - 12.1 *(Latimer et al., 2003)*	8 – 11 *(Reece, 2004)*
Neu	56.4 ± 5.750	38 – 70 *(Hadaybit Laboratory (UAE))*	52 – 70 *(Latimer et al., 2003)*	50 – 60 *(Reece, 2004)*
Lym	36.64 ± 6.237	25 – 62 *(Hadaybit Laboratory (UAE))*	21 – 42 *(Latimer et al., 2003)*	30 – 40 *(Reece, 2004)*
Mon	2.63 ± 1.330	1 – 8 *(Hadaybit Laboratory (UAE))*	0 – 6 *(Latimer et al., 2003)*	5 – 6 *(Reece, 2004)*
Eos	1.22 ± 0.654	0.1 – 9.5 *(Hadaybit Laboratory (UAE))*	0 – 7 *(Latimer et al., 2003)*	2 – 5 *(Reece, 2004)*
Bas	0.11 ± 0.538	0 – 1.2 *(Hadaybit Laboratory (UAE))*	0 – 2 *(Latimer et al., 2003)*	<1 *(Reece, 2004)*

Ery: Erythrocyte(x 10^{12}g/l); **Hgb:** Haemoglobin (g/dl); **Leu:** Leukocyte(x 10^{9}/l); **Neu:** Neutrophil (%); **Lym:** Lymphocyte (%); **Mon:** Monocyte (%); **Eos:** Eosinophil (%); **Bas:** Basophil (%). **LSMean:** least square mean. (*Reference indicated in italics).*

4.1.4 Basal blood metabolites

The mean creatinine concentration (1.11 ± 0.144 mg/dl) of the horses in the study was well within the reference range provided by Hadaybit Laboratory (UAE), Reece (2004), Latimer and Prasse (2003) and Orsini *et al*. (2008). Creatinine is a nitrogenous by-product of muscle metabolism. Any creatinine generated when muscles are broken down, will end up in the circulatory system. As creatinine is not reused by the body, and is eventually excreted, it is a useful indication of renal function. As the mean creatinine concentration is well within the reference range, it follows that the overall kidney function of the group of horses used in this trial was normal.

Another indicator of renal function is blood urea nitrogen (BUN). The liver produces urea during the urea cycle as a waste product of protein digestion. Urea is transported to the blood, and excreted by the kidneys. The ability of the kidneys to remove the urea from the blood is evaluated when measuring the BUN concentration. The mean BUN concentration for all horses over the entire trial period (23.82 ± 4.578 mg/dl) is at the higher end of the reference range as provided by Hadaybit Laboratory (UAE), Reece (2004),

Latimer and Prasse (2003) and Orsini *et al*. (2008). This could be due to a high protein turnover of the horses' muscles as a result of high exercise intensities before start of trial.

Lactate dehydrogenase (LDH) is the enzyme responsible for breaking down lactate. Mean LDH concentration (570.19 ± 136.930 U/l) was slightly above that of the reference range reported by Hadaybit Laboratory (UAE), Latimer and Prasse (2003) and Orsini *et al*. (2008). As these were fit, hardworking horses at the end of a racing season, they were able to effectively break down lactate in order to recover from strenuous exercise. It can thus be expected that the LDH values will be above the reference range for average horses (not adapted to this intensity of exercise).

Mean lactate concentration (0.84 ± 0.219 mmol/l) of these horses is slightly below the reference range reported by Hadaybit Laboratory (UAE), Reece (2004) and Orsini *et al*. (2008). This is probably because the horses were fit and had elevated LDH concentrations, which led to the horses being able to break lactate down more effectively.

Mean cholesterol concentration (97.41 ± 10.656 mg/dl) was well within the reference range reported by Reece (2004), Latimer and Prasse (2003) and CVR Laboratory. Two components of cholesterol are high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

Mean HDL (72.41 ± 9.817 mg/dl) and LDL (27.49 ±7.327 mg/dl) concentrations were well within the reference range provided by Hadaybit Laboratory (UAE). Total cholesterol, HDL and LDL are all indicators of energy metabolism. As all of these values were well within the reference range, it can be concluded that the overall energy metabolism of these horses was normal.

Summary statistics of physiological blood metabolites for horses included in this study are provided in Table 4.1.4.

Table 4.1.4 Summary statistics of physiological blood metabolites for endurance horses compared to reference range

	LSMean ± SD	Reference range	Reference range	Reference range	Reference range	Reference range
Creatinine	1.11 ± 0.144	0.9 – 2.0 *(Hadaybit Laboratory (UAE))*	1 – 2 *(Reece 2004)*	0.4- 2.2 *(Latimer & Prasse, 2003)*	0.9 – 1.9 *(Orsini et al., 2008)*	
LDH	570.19 ± 136.930	160 – 500 *(Hadaybit Laboratory (UAE))*		112 – 456 *(Latimer & Prasse, 2003)*	162 – 412 *(Orsini et al., 2008)*	
Lactate	0.84 ± 0.219	0.9 – 1.7 *(Hadaybit Laboratory (UAE))*	1.11 – 1.78 *(Reece 2004)*		1.11 – 1.78 *(Orsini et al., 2008)*	
BUN	23.82 ± 4.578	10 – 24 *(Hadaybit Laboratory (UAE))*	10 – 24 *(Reece 2004)*	11- 27 *(Latimer & Prasse, 2003)*	12 – 24 *(Orsini et al., 2008)*	
Cholesterol	97.41 ± 10.656	71 – 142 *(Hadaybit Laboratory (UAE))*	75 – 150 *(Reece 2004)*	71 – 142 *(Latimer & Prasse, 2003)*	75 – 150 *(Orsini et al., 2008)*	70 – 170 *(CVR Laboratory)*
HDL	72.41 ± 9.817	30 – 90 *(Hadaybit Laboratory (UAE))*				
LDL	27.49 ±7.327	20 – 40 *(Hadaybit Laboratory (UAE))*				

Creatinine: mg/dl; **LDH:** Lactate dehydrogenase (U/l)**; Lactate:** mmol/l**; BUN:** Blood urea nitrogen (mg/dl)**; Cholesterol:** mg/dl; **HDL:** High density lipoprotein (mg/dl); **LDL**: Low density lipoprotein (mg/dl). **LSMean:** least square mean. (*Reference indicated in italics).*

4.1.5 Basal blood glucose and specific hormone concentrations

Mean glucose concentration (111.97 ± 15.931 mg/dl) for all horses was at the higher end of the reference range provided by Hadaybit Laboratory (UAE) and Orsini *et al*. (2008); however, it was well within the reference range reported by Latimer and Prasse (2003). As the blood samples were collected post parandially, it could be the reason for the higher than normal blood glucose concentrations observed.

Mean cortisol concentration (101.78 ± 27.894 nmol/l) was at the lower end of the reference range reported by Hadaybit and CVR Laboratory. This indicates that the horses had low long-term stress levels, and were well adapted to their environment.

Average T3 concentration for all the horses (0.65 ± 0.204 nmol/l) was at the lower end of the reference range provided by Idexx Laboratory (RSA), CVR Laboratory and Robinson and Sprayberry (2009).

Mean T4 concentration for all the horses included in the study (32.52 ± 8.434 nmol/l) was at the higher end of the reference range reported by Idexx Laboratory (RSA) and Robinson and Sprayberry (2009). However, the mean T4 concentration was higher than the reference range provided by CVR Laboratory. T4 (Thyroxin) is the less active of the two hormones that regulate metabolic rate, and serves as the precursor of T3 (Triiodothyronine). T3 is the physiologically more important of the two hormones and is present in much higher blood concentrations than T4.

Mean insulin concentration for all horses in this study (5.63 ± 4.991 µlU/l) was at the lower end of the reference range provided by Idexx Laboratory (RSA); however, it was below the reference range reported by Orsini *et al*. (2008) and CVR Laboratory. As insulin is quite unstable, it is possible that some of the insulin was broken down prior to analysis, and these results should be interpreted with caution.

Summary statistics of blood glucose and specific hormone concentrations for horses included in this study are provided in Table 4.1.5.

Table 4.1.5 Summary statistics of blood glucose and specific hormone concentrations for endurance horses compared to reference range

	LSMean ± SD	Reference range	Reference range	Reference range	Reference range
Glucose	111.97 ± 15.931	62 – 114 *(Hadaybit Laboratory (UAE))*	75 – 115 *(Orsini et al., 2008)*		62 – 134 *(Latimer & Prasse, 2003)*
Cortisol	101.78 ± 27.894	90 – 200 *(Idexx Laboratory (RSA))*		69 – 180 *(CVR Laboratory)*	
T3	0.65 ± 0.204	0.5 – 2.5 *(Idexx Laboratory (RSA))*		0.24 – 1.28 *(CVR Laboratory)*	0.3 – 2.9 *(Robinson & Sprayberry, 2009)*
T4	32.52 ± 8.434	11 – 37 *(Idexx Laboratory (RSA))*		16.5 – 24.4 *(CVR Laboratory)*	6 – 46 *(Robinson & Sprayberry, 2009)*
Insulin	5.63 ± 4.991	4 – 20 *(Idexx Laboratory (RSA))*	10 – 30 *(Orsini et al., 2008)*	5.7 – 31.4 *(CVR Laboratory)*	

Glucose: mg/dl; **Cortisol:** nmol/l; **T3:** Triiodothyronine (nmol/l)**; T4:** Thyroxin (nmol/l); **Insulin:** (µlU/l). **LSMean:** least square mean. (*Reference indicated in italics).*

4.2. Pooled results for the four treatment groups from Day 0 to Day 19

These results include combined data for both the adaptation phase (day 0 – day 9) as well as the supplementation period (day 10 – day 19).

4.2.1 Pre-exercise basal physiological parameters

Basal heart rate

The CLA treatment group (31.5 ± 5.07 bpm) and the control group (31.0 ± 4.97 bpm) did not differ significantly from each other with regard to basal heart rate (beats per minute/bpm), although both differed significantly (P <0.05) from the Schisandra (27.6 ± 4.55 bpm) and the combination (29.0 ± 4.43 bpm) treatment groups. Heart rates of horses in the Schisandra and combination treatment groups did not differ significantly from each other. Mean basal heart values of the four different groups were within the reference range reported by Huntington *et al*. (2004), Detweiler and Erickson (2004) and Evans (1994).

The lower basal heart rate of the Schisandra and combination treatment groups compared to the CLA and control group indicates that Schisandra (supplemented on its own or in combination with CLA) had a significant effect on lowering the basal heart rate in horses. Hancke *et al*. (1994) found similar results.

It is possible that Schisandra increased both the oxygen supply to the muscles and carbon dioxide (and other waste products) removal from the muscles. Both these effects result in a more efficient circulatory system, and protects the heart by reducing strain as a result of overworking. This could be very beneficial in endurance racing, as a minimum heart rate has to be achieved by the horses during a race in order for them to pass the vet checks and thus continue in the race.

Trendlines for these results are presented in Figure 4.2.1.

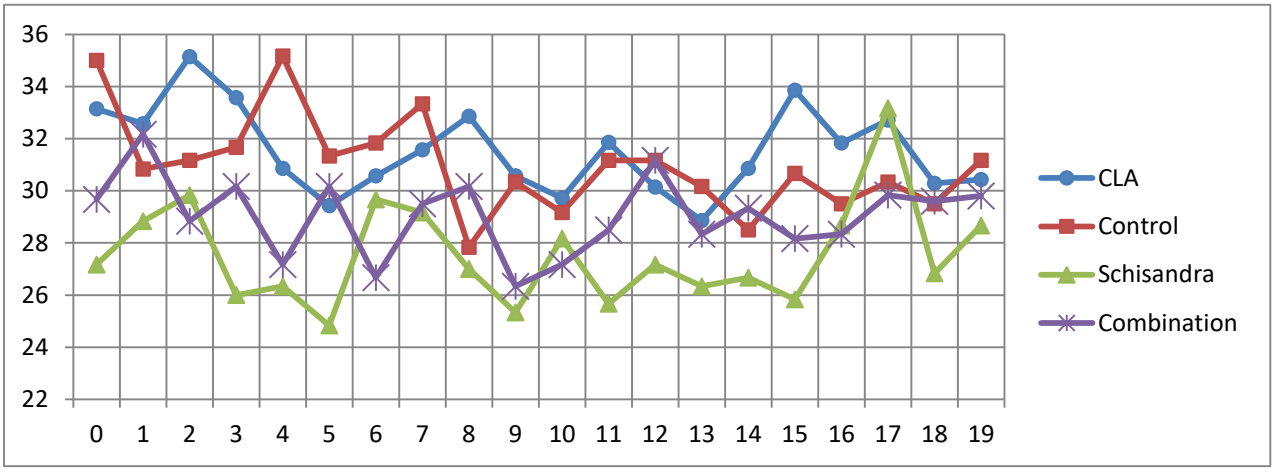

Figure 4.2.1 Changes in basal heart rate (beats per minute) of the four treatment groups over time

Basal respiration rate

The only significant (P <0.05) difference observed in terms of basal respiration rate (inhalations per minute/ipm) between the treatment groups was between the CLA group (12.3 ± 3.00 ipm) and the control group (11.2 ±2 .85 ipm). The CLA group did not differ significantly from the Schisandra group (12.1 ± 3.27 ipm) or the combination group (11.6 ± 3.69 ipm). The control group also did not differ significantly from the Schisandra or combination treatment groups.

CLA increased (P <0.05) the basal respiration rate in the endurance horses (when supplemented on its own) compared to the control group. This is probably due to an increased metabolic rate as found by Kamphuis *et al*. (2003) (in humans), as the CLA also slightly increased the basal heart rate (this effect was not significant).

Trendlines for these results are presented in Figure 4.2.2.

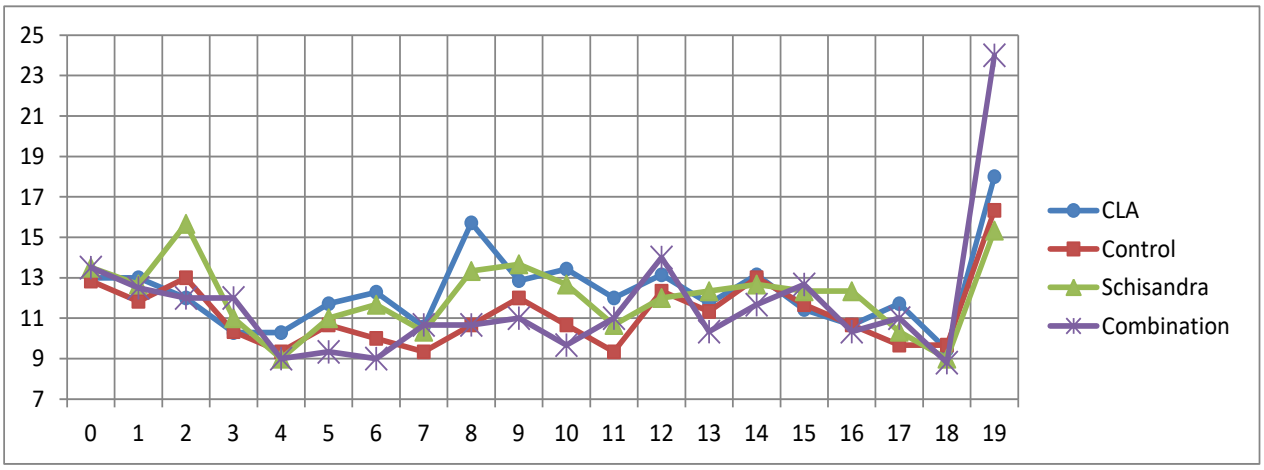

Figure 4.2.2 Changes in basal respiration rate (inhalations per minute) of the four treatment groups over time

Basal temperature (rectal)

The Schisandra treatment group (36.5 ± 0.65 °C) differed significantly (P <0.05) from both the control (36.7 ± 0.52 °C) and CLA (36.7 ± 0.60 °C) treatment groups in terms of basal rectal temperature. The Schisandra group did not differ significantly from the combination treatment group (36.6 ± 0.55 °C). Rectal temperature of all four groups was slightly below the reference range reported by Huntington *et al*. (2004) (37.0 – 38.0 °C) and Robertshaw (2004) (37.2 – 38.2 °C). This could be because of the cool winter climate experienced during the study and early morning temperature recordings.

From this we see that Schisandra has a definite effect on lowering rectal temperature (although the drop in rectal temperature was small). According to Lupandin *et al*. (1986), *Schisandra chinensis* is an adaptogen, and protects against various stressors, including heat stress. This could be of benefit to horses in warmer climates, helping them to successfully thermoregulate.

Trendlines for these results are presented in Figure 4.2.3.

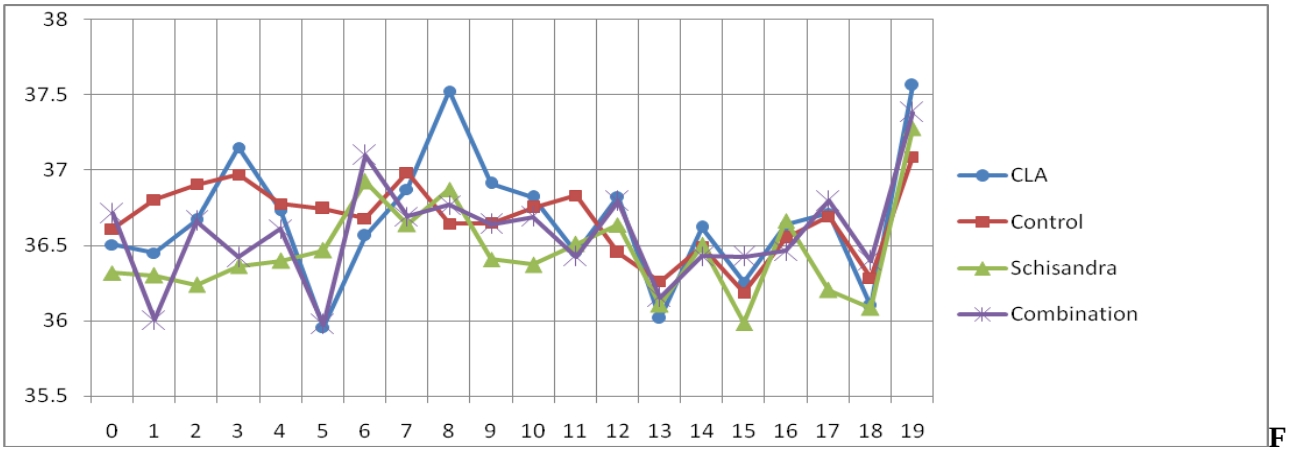

Figure 4.2.3 Changes in basal rectal temperature (degrees Celsius) of the four treatment groups over time

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 4.2.1.

Table 4.2.1 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control - Pooled results (reference range for adult horses also indicated with reference in italics)

	Basal HR LSMean ± SD	Basal Resp LSMean ± SD	Basal Temp LSMean ± SD
CLA **(n=139)**	31.5[a] ±5.07	12.3[a] ±3.00	36.7[a] ±0.60

Control (n=120)	31.0[a] ±4.97	11.2[b] ±2.85	36.7[a] ±0.52
Schisandra (n=120)	27.6[b] ±4.55	12.1[ab] ±3.27	36.5[b] ±0.65
Combination (n=118)	29.0[b] ±4.43	11.6[ab] ±3.69	36.6[ab] ±0.55
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington et al., 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.2.2 Post exercise parameters

Heart rate 0 minutes post exercise

No significant differences were found between the four treatment groups in terms of heart rate at 0 minutes post exercise (pooled results).

Heart rate 10 minutes post exercise

Schisandra treatment group (28.7 ± 4.20 bpm) differed significantly (P <0.05) from both the CLA (31.9 ± 3.30 bpm) and the control (31.8 ± 5.09 bpm) groups with regards to heart rate at 10 minutes post exercise. The Schisandra group did not differ significantly from the combination treatment group (30.2 ± 3.50 bpm). No other significant differences were found.

Once again, it is clear that Schisandra had an effect on lowering heart rate values. This was also the findings of Ahumada *et al*. (2006). It is possible that Schisandra increased both the oxygen supply to the muscles and carbon dioxide (and other waste products) removal from the muscles. Both these effects result in a more efficient circulatory system, and protects the heart by reducing strain as a result of overworking. This could be very beneficial in endurance racing, as a minimum heart rate has to be achieved by the horses during a race in order for them to pass the vet checks and thus continue in the race.

Heart rate 20 minutes post exercise

At 20 minutes post exercise, the heart rate values of the Schisandra group (28.1 ± 5.09 bpm) differed significantly (P <0.05) from both the CLA (31.2 ± 4.31 bpm) and the control (30.9 ± 4.95 bpm) treatment

groups. Once again, the Schisandra group did not differ significantly from the combination treatment group (30.61 ±2.73 bpm). No other significant differences were found.

The heart rate lowering effect of Schisandra is apparent from 10 minutes post exercise until 20 minutes. As no measures were recorded after 20 minutes post exercise, it is not possible to determine how long this effect lasts. The addition of CLA to the Schisandra supplements (as in the combination treatment group) reduces the heart rate lowering effect of Schisandra. This could be due to the fact that CLA increases metabolism (Kamphuis *et al*., 2003), which results in an increased heart rate.

Respiration rate 0 minutes post exercise

The respiration rate at 0 minutes post exercise of the CLA treatment group (24.1 ± 6.66 ipm) differed significantly (P <0.05) only from the combination treatment group (29.7 ± 6.39 ipm). The control group (21.1 ± 8.72 ipm) differed significantly (P <0.05) from both the Schisandra group (27.1 ± 8.46 ipm), as well as the combination group. The Schisandra and combination groups did not differ significantly from each other.

The combination of the CLA and Schisandra (combination group) seemed to have increased the respiration rate at 0 minutes post exercise above that of which each supplement would have if fed separately. All groups which received a supplement (CLA, Schisandra or combination) displayed higher respiration rates at 0 minutes post exercise compared to the control group, although not all of these were significant at the 95% level. It has been known for a long time that Schisandra stimulates respiration, improving gas exchange in both the lungs and the cells of the body (Konstantinov, 1959). The mechanism of this is unclear, however it is believed to be due to an increased efficiency of oxygen utilization. More studies are required to confirm these assumptions. The increase in respiration rate observed in the CLA group is most probably due to the stimulating effect of CLA on the metabolic rate (Kamphuis *et al*., 2003), which results in an increased respiration rate.

Respiration rate 10 minutes post exercise

With respiration rate at 10 minutes post exercise, both the CLA (23.1 ± 7.48 ipm) and Schisandra (22.9 ± 7.31 ipm) treatment groups differed significantly (P <0.05) from the control group (18.0 ± 8.43 ipm), but not from each other. The combination group (22.1 ± 7.69 ipm) did not differ significantly from any other group.

An increase in respiration was observed in the groups which received supplements (CLA, Schisandra or combination) compared to the control group, although only the CLA and Schisandra groups showed significant (P <0.05) increases in respiration rate 10 minutes post exercise. The magnitude of the increase in respiration of the combination group is much smaller 10 minutes post exercise in comparison to 0 minutes

post exercise. In fact, this increase is not significantly different from any of the other groups (CLA, control or Schisandra) at 10 minutes post exercise.

Respiration rate 20 minutes post exercise

	HR 0 min ±SD	HR 10 min ±SD	HR 20 min ±SD	Resp 0 min ±SD	Resp 10 min ±SD	Resp 20 min ±SD
CLA (n=34)	33.7 ±4.25	31.9[a] ±3.30	31.2[a] ±4.31	24.1[ac] ±6.66	23.1[a] ±7.48	23.0[a] ±5.65

The control group (16.8 ± 7.51 ipm) differed significantly (P <0.05) from all the other treatment groups in terms of respiration rate 20 minutes post exercise. The CLA (23.0 ± 5.65 ipm), Schisandra (21.5 ± 7.82 ipm) and combination (21.5 ± 6.15 ipm) treatment groups did not differ significantly from each other.

An increased respiration rate was again observed in the groups which received supplements (CLA, Schisandra or combination).The CLA, Schisandra and combination supplements fed to the horses can be said to increase respiration rate at 0, 10 and 20 minutes after exercise (not all significantly so). It is possible that this is due to an increased metabolic rate brought on by the different supplements. It has been known for a long time that CLA stimulates the metabolism (Kamphuis *et al*., 2003), resulting in an increased metabolic rate, whereas S*chisandra chinensis* has been reported to stimulate respiration (Konstantinov, 1959), increasing oxygen intake and uptake. The mechanism of this is unclear, however it is believed to be due to an increased efficiency of oxygen utilization. More studies are required to confirm these assumptions.

Post exercise parameters of the four groups of horses included in this study are summarized in Table 4.2.2.

Table 4.2.2. Least square means (LSMeans) and standard deviations (SD) of post exercise parameters for endurance horses that received dietary supplements compared to a negative control - Pooled results

Control (n=30)	34.2 ±3.74	31.8^{a} ±5.09	30.9^{a} ±4.95	21.1^{a} ±8.72	18.0^{b} ±8.43	16.8^{b} ±7.51
Schisandra (n=30)	31.7 ±5.32	28.7^{b} ±4.20	28.1^{b} ±5.09	27.1^{bc} ±8.46	22.9^{a} ±7.31	21.5^{a} ±7.82
Combination (n=28)	33.0 ±3.61	30.2^{ab} ±3.50	30.6^{ab} ±2.73	29.7^{b} ±6.39	22.1^{ab} ±7.69	21.5^{a} ±6.15

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm) 20 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. LSMeans with different superscript letters in the same columns differed (P <0.05).

4.2.3 Haematological values

Blood pH

No significant differences were found between the four treatment groups in terms of blood pH (pooled results).

Erythrocyte count

Erythrocyte count of the CLA treatment group (7.12 ± 0.530 x 10^{12}g/l) differed significantly (P <0.05) from both that of the control (6.31 ± 0.485 x 10^{12}g/l) and Schisandra group (6.50 ± 0.453 x 10^{12}g/l). The control and Schisandra group did not differ significantly from each other. The combination treatment group (6.79 ± 0.635 x 10^{12}g/l) did not differ significantly from any other group. Compared to the control group, the combination group had higher erythrocyte values, although not significantly so.

From these results it seems possible that CLA stimulated splenic contractions, resulting in a release of erythrocytes into the blood stream (when used as a supplement on its own or with Schisandra).

Haemoglobin (Hgb)

Haemoglobin concentration of the control group (11.20 ± 0.746 g/dl) differed significantly (P <0.05) from both the CLA group (12.15 ± 0.742 g/dl) and the combination group (12.09 ± 1.099 g/dl), but not from the Schisandra group (11.43 ± 0.942 g/dl). No other significant differences between the groups were found with regards to haemoglobin concentrations.

From these results it is clear that CLA stimulated splenic contractions, resulting in a release of erythrocytes into the blood stream, which increased the haemoglobin concentrations in the blood. Increased haemoglobin concentrations in the blood would result in increased performance, as more oxygen would reach the muscles,

increasing the muscle capacity to do aerobic work. This is also reported by Lupandin *et al*. (1986). Anaerobic respiration would also decrease if more oxygen reached the muscles, resulting in less lactic acid production.

Leukocyte count

The only significant (P <0.05) difference found in terms of leukocyte concentration was between the CLA treatment group (6.83 ± 1.548 x 10^9/l) and the Schisandra treatment group (5.36 ± 0.679 x 10^9/l). The control group (6.33 ± 0.940 x 10^9/l) did not differ significantly from the combination group (6.18 ± 1.114 x 10^9/l), or any other group.

Multiple factors influence leukocyte production and concentrations; thus, these results should be interpreted with caution.

Differential blood count

No significant differences were found between the four treatment groups in terms of neutrophil, lymphocyte, monocyte, eosinophil and basophil counts.

Haematological values of the four groups of horses included in this study are summarized in Table 4.2.3.

Table 4.2.3 Least square means (LSMeans) and standard deviations (SD) of haematological values for endurance horses that received dietary supplements compared to a negative control - Pooled results (reference range for adult horses also indicated with reference in italics)

	Blood pH LSMeans ±SD	Ery ±SD	Hgb ±SD	Leu ±SD	Neu ±SD	Lym ±SD	Mon ±SD	Eos ±SD	Bas ±SD
CLA (n= 21)	7.55 ±0.038	7.12[a] ±0.530	12.15[a] ±0.742	6.83[a] ±1.548	58.28 ±4.489	37.56 ±5.487	2.89 ±1.916	1.28 ±0.467	0.00 ±0.000
Control (n=18)	7.51 ±0.035	6.31[b] ±0.485	11.20[b] ±0.746	6.33[ab] ±0.940	56.89 ±8.139	39.46 ±8.527	2.53 ±0.830	1.09 ±0.614	0.03 ±0.118
Schisandra (n= 18)	7.52 ±0.090	6.50[b] ±0.453	11.43[ab] ±0.942	5.36[b] ±0.679	55.34 ±4.874	40.87 ±5.397	2.42 ±1.111	1.16 ±0.784	0.22 ±0.919
Combination (n=17)	7.54 ±0.036	6.79[ab] ±0.635	12.09[a] ±1.099	6.18[ab] ±1.114	54.68 ±4.551	41.09 ±4.693	2.66 ±1.135	1.36 ±0.758	0.21 ±0.599
Reference range	7.5 – 8.5 *(Hadaybit Laboratory (UAE))*	4.8 – 13 *(Hadaybit Laboratory (UAE))*	9 – 15 *(Hadaybit Laboratory (UAE))*	5 – 11 *(Hadaybit Laboratory (UAE))*	38 – 70 *(Hadaybit Laboratory (UAE))*	25 – 62 *(Hadaybit Laboratory (UAE))*	1 – 8 *(Hadaybit Laboratory (UAE))*	0.1 – 9.5 *(Hadaybit Laboratory (UAE))*	0 – 1.2 *(Hadaybit Laboratory (UAE))*
Reference range		6.0 – 10.4 *(Latimer et al., 2003)*	10 – 16 *(Latimer et al., 2003)*	5.6 - 12.1 *(Latimer et al., 2003)*	52 – 70 *(Latimer et al., 2003)*	21 – 42 *(Latimer et al., 2003)*	0 – 6 *(Latimer et al., 2003)*	0 – 7 *(Latimer et al., 2003)*	0 – 2 *(Latimer et al., 2003)*
Reference range	7.35 – 7.45 *(Davies, 2009)*	7 - 11 *(Reece, 2004)*	11.5 - 16 *(Reece, 2004)*	8 - 11 *(Reece, 2004)*	50 - 60 *(Reece, 2004)*	30 - 40 *(Reece, 2004)*	5 - 6 *(Reece, 2004)*	2 - 5 *(Reece, 2004)*	<1 *(Reece, 2004)*

Ery: Erythrocyte(x 10^{12}g/l); **Hgb:** Haemoglobin (g/dl); **Leu:** Leukocyte (x 10^{9}/l); **Neu:** Neutrophil (%); **Lym:** Lymphocyte (%); **Mon:** Monocyte (%); **Eos:** Eosinophil (%); **Bas:** Basophil (%). (*Reference indicated in italics)***.** LSMeans with different superscript letters in the same columns differed ($p<0.05$).

4.2.4 Blood metabolites

Creatinine

No significant differences were found between the four treatment groups in terms of creatinine concentrations (pooled results).

Lactate

No significant differences were found between the four treatment groups in terms of lactate concentrations. The mean lactate concentrations of the CLA, control and Schisandra groups were below the reference range as reported by Hadaybit Laboratory (UAE), Orsini *et al*. (2008) and Reece (2004). This could be due to elevated LDH concentrations (the enzyme responsible for clearing lactate from the system).

Lactate Dehydrogenase (LDH)

LDH concentration of the control group (471.78 ±102.941 U/l) differed significantly (P <0.05) from that of the CLA (613.95 ±142.720U/l) and combination (672.71 ±128.817U/l) groups, but not from the Schisandra group (520.72 ±±67.932U/l). The CLA and combination groups did not differ significantly from each other. Similarly, the Schisandra group did not differ significantly from the CLA group; however, a significant (P <0.05) difference was found between Schisandra and the combination group. The LDH values of the control group fell within the reference range, whereas the other three groups' LDH values were above the reference range reported by Hadaybit Laboratory (UAE), Latimer and Prasse (2003) and Orsini *et al*. (2008). It is possible that both the Schisandra and CLA stimulated LDH production.

CLA, Schisandra and combination treatment groups showed an increase in the enzyme concentrations above the control group (although the increase in the Schisandra group was not significant), as well as the reference range. This increase was especially apparent (and significant: P <0.05) in the groups who received CLA supplements (CLA group and the combination group) in comparison to the control group. From this it can be concluded that CLA increased the LDH concentrations in blood. LDH is an enzyme responsible for clearing lactic acid from the body. Horses with higher LDH concentrations in their blood would be able to clear lactic acid from their system more effectively, which could improve performance, as well as aid in the recovery of the body after strenuous exercise.

Blood urea nitrogen (BUN)

The only significant (P <0.05) difference with regards to blood BUN concentration was between the combination group (22.74 ±4.442 mg/dl) and the CLA group (24.61 ±4.726 mg/dl). The blood BUN concentrations of the control (24.14 ±4.602 mg/dl) and Schisandra (23.60 ±4.677 mg/dl) groups did not differ significantly from each other or any other group.

The lower BUN value observed in the combination group (compared to the CLA group) could suggest improved digestion of protein and/or enhanced renal function. As CLA is present in the supplements of both of these groups (CLA group and combination group), it cannot solely be responsible for the lower BUN values in the combination group. It could be that Schisandra (present in the combination group) is responsible for these lower BUN concentrations in blood (the BUN level in the Schisandra group was also lower than that of both the control and CLA groups, although not significantly so).

Total cholesterol

The combination group (106.00 ±10.548 mg/dl) displayed significantly (P <0.05) higher total cholesterol values than any other group. The mean cholesterol concentrations of the CLA (96.67 ±7.255 mg/dl), control (91.44 ±12.524 mg/dl) and Schisandra (96.11 ±7.087 mg/dl) groups did not differ significantly from each other.

It seems as if the combination of CLA and Schisandra has an additive effect in increasing cholesterol concentrations in horses, as neither the CLA nor the Schisandra increased cholesterol levels significantly when supplemented separately. This is possibly due to the increased amounts of energy substrates made available to the body by the different supplements.

High density lipoprotein (HDL)

Again the combination group (83.06 ±6.851 mg/dl) displayed significantly (P <0.05) higher HDL values than any other group.

Blood metabolite concentrations of the four groups of horses included in this study are summarized in Table 4.2.4.

Table 4.2.4 Least square means (LSMeans) and standard deviations (SD) of blood metabolites for endurance horses that received dietary supplements compared to a negative control - Pooled results (reference range for adult horses also indicated with reference in italics)

	Creatinine ± SD	**Lactate ± SD**	**LDH ± SD**	**BUN ± SD**	**Cholesterol ± SD**	**HDL ± SD**	**LDL ± SD**
CLA (n=21)	1.13 ±0.104	0.78 ±0.248	613.95^{ac} ±142.720	24.61^{a} ±4.726	96.67^{a} ±7.255	70.33^{a} ±6.240	29.62 ±7.003
Control (n=18)	1.07 ±0.132	0.86 ±0.212	471.78^{b} ±102.941	24.14^{ab} ±4.602	91.44^{a} ±12.524	67.94^{a} ±10.952	25.72 ±5.389
Schisandra (n=18)	1.14 ±0.199	0.82 ±0.215	520.72^{ab} ±67.932	23.60^{ab} ±4.677	96.11^{a} ±7.087	69.22^{a} ±7.305	27.89 ±5.592
Combination (n=17)	1.08 ±0.130	0.91 ±0.186	672.71^{c} ±128.817	22.74^{b} ±4.442	106.00^{b} ±10.548	83.06^{b} ±6.851	26.29 ±10.409
Reference range	0.9 – 2.0 *(Hadaybit Laboratory (UAE))*	0.9 – 1.7 *(Hadaybit Laboratory (UAE))*	160 – 500 *(Hadaybit Laboratory (UAE))*	10 – 24 *(Hadaybit Laboratory (UAE))*	71 – 142 *(Hadaybit Laboratory (UAE))*	30 – 90 *(Hadaybit Laboratory (UAE))*	20 – 40 *(Hadaybit Laboratory (UAE))*
Reference range	0.4 – 2.2 *(Latimer and Prasse, 2003)*		112 – 456 *(Latimer and Prasse, 2003)*	11 – 27 *(Latimer and Prasse, 2003)*	71 – 142 *(Latimer and Prasse, 2003)*		
Reference range	0.9 – 1.9 *(Orsini et al., 2008)*	1.11 – 1.78 *(Orsini et al., 2008)*	162 – 412 *(Orsini et al., 2008)*	12 – 24 *(Orsini et al., 2008)*	75 – 150 *(Orsini et al., 2008)*		
Reference range	1 – 2 *(Reece, 2004)*	1.11 – 1.78 *(Reece, 2004)*		10 – 24 *(Reece, 2004)*	70 – 170 *(CVR Laboratory)*		

Creatinine: mg/dl; **Lactate:** mmol/l**; LDH:** Lactate dehydrogenase (U/l)**; BUN:** Blood urea nitrogen (mg/dl)**; Cholesterol:** mg/dl; **HDL:** High density lipoprotein (mg/dl); **LDL**: Low density lipoprotein (mg/dl)**.** (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.2.5 Blood glucose and specific hormone concentrations

Glucose

The only significant (P <0.05) difference in terms of glucose concentration was between the CLA group (105.76 ±10.251 mg/dl) and the control group (116.39 ±19.473 mg/dl). The Schisandra (115.33 ±19.545 mg/dl) and combination (111.41 ±11.500 mg/dl) groups did not differ significantly from any other group.

The lower glucose level in the CLA group, compared to the control group, could indicate a better uptake (and use) of glucose from the blood due to an increased rate of metabolism brought on by the CLA supplement (Kamphuis *et al*., 2003). CLA also reduces insulin insensitivity, which would result in an increased uptake of glucose from the blood. The combination group (which contained CLA supplement) showed a lower blood glucose level than the control group, but this was not significant.

Cortisol, T3 and Insulin

No significant differences were found between the four treatment groups in terms of cortisol, T3 or insulin concentrations (pooled results).

Thyroxin (T4)

In terms of T4 concentrations, the combination (27.44 ±6.849 nmol/l) group differed significantly (P <0.05) from both the control (36.44 ±8.864 nmol/l) and the Schisandra (36.25 ±7.680 nmol/l) treatment groups. However, the control and Schisandra treatment groups did not differ significantly from each other. The CLA group (30.08 ±7.114 nmol/l) did not differ significantly from any other group with regard to T4 concentrations.

It seems as if CLA had an effect on lowering blood T4 concentrations, especially when fed in combination with Schisandra. It could be that the combination of CLA and Schisandra stimulates the conversion of T4 (physiologically less active) to T3 (physiologically more active), in order to increase metabolic rate.

Blood glucose and specific hormone concentrations of the four groups of horses included in this study are summarized in Table 4.2.5.

Table 4.2.5 Least square means (LSMeans) and standard deviations (SD) of blood glucose and specific hormone concentrations for endurance horses that received dietary supplements compared to a negative control - Pooled results (reference range for adult horses also indicated with reference in italics)

	Glucose ±SD	**Cortisol ±SD**	**T3 ±SD**	**T4 ±SD**	**Insulin ±SD**
CLA (n=21)	105.76[a] ±10.251	98.48 ±21.763	0.68 ±0.200	30.08[ab] ±7.114	5.72 ±3.548
Control (n=18)	116.39[b] ±19.473	102.20 ±28.401	0.68 ±0.309	36.44[a] ±8.864	6.08 ±5.878
Schisandra (n=18)	115.33[ab] ±19.545	103.27 ±32.219	0.61 ±0.145	36.25[a] ±7.680	5.78 ±6.157
Combination (n=17)	111.41[ab] ±11.500	103.82 ±31.250	0.61 ±0.097	27.44[b] ±6.849	4.90 ±4.485
Reference range	75 – 115 *(Orsini et al., 2008)*				10 – 30 *(Orsini et al., 2008)*
Reference range		69 – 180 *(CVR Laboratory)*	0.24 – 1.28 *(CVR Laboratory)*	16.5 – 24.4 *(CVR Laboratory)*	5.7 – 31.4 *(CVR Laboratory)*
Reference range	62 – 114 *(Hadaybit Laboratory (UAE))*	90 – 200 *(Idexx Laboratory (RSA))*	0.5 – 2.5 *(Idexx Laboratory (RSA))*	11 – 37 *(Idexx Laboratory (RSA))*	4 – 20 *(Idexx Laboratory (RSA))*
Reference range	62 – 134 *(Latimer and Prasse, 2003)*		0.3 – 2.9 *(Robinson & Sprayberry, 2009)*	6 – 46 *(Robinson & Sprayberry, 2009)*	

Glucose: mg/dl; **Cortisol:** nmol/l; **T3:** Triiodothyronine (nmol/l)**; T4:** Thyroxin (nmol/l); **Insulin:** (µlU/l). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.3. Treatment phase results for Day 10 to Day 19

Results for the supplementation period is presented below.

4.3.1 Pre-exercise basal physiological parameters

Basal heart rate

Basal heart rate (beats per minute/bpm) of the CLA treatment group (31.0 ± 5.23 bpm) differed significantly (P <0.05) from both the Schisandra (27.7 ± 3.92 bpm) and combination (29.0 ± 3.43 bpm) treatment groups, but not from the control group (30.1 ± 3.96 bpm). The control group differed significantly (P <0.05) from the Schisandra group, but not from the combination group in terms of basal heart rate. The basal heart rate of Schisandra and combination groups did not differ significantly.

The lower basal heart rate of the Schisandra and combination treatment groups compared to the CLA and control group indicates that Schisandra (supplemented on its own or in combination with CLA) has a significant effect on lowering the basal heart rate in horses. It is possible that Schisandra increased both the oxygen supply to the muscles and carbon dioxide (and other waste products) removal from the muscles. Both these effects result in a more efficient circulatory system, and protects the heart by reducing strain as a result of overworking. This could be very beneficial in endurance racing, as a minimum heart rate has to be achieved by the horses during a race in order for them to pass the vet checks and thus continue in the race.

Basal respiration rates

No significant differences were found between the four treatment groups in terms of basal respiration rates (inhalations per minute/ipm) during the treatment phase.

Basal temperature (rectal)

No significant differences were found between the four treatment groups in terms of basal rectal temperatures during the treatment phase. All four groups' rectal temperature was slightly below the reference range reported by Huntington *et al*. (2004) and Robertshaw (2004). This could be because of the cool winter climates and early morning temperature recordings.

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 4.3.1.

Table 4.3.1 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control – Treatment phase results (reference range for adult horses also indicated with reference in italics)

	Basal HR ±SD	**Basal Resp ±SD**	**Basal Temp ±SD**
CLA (n=69)	31.0[a] ±5.23	12.5 ±3.24	36.6 ±0.59
Control (n=60)	30.1[ac] ±3.96	11.5 ±3.37	36.6 ±0.50
Schisandra (n=60)	27.7[b] ±3.92	12.0 ±3.42	36.4 ±0.70
Combination (n=58)	29.0[bc] ±3.43	12.2 ±4.44	36.6 ±0.49
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington et al., 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.3.2 Post exercise parameters

Heart rate 0 minutes post exercise

No significant differences were found between the four treatment groups in terms of heart rate at 0 minutes post exercise during the treatment phase.

Heart rate 10 minutes post exercise

The Schisandra treatment group (28.6 ± 2.97 bpm) differed significantly (P <0.05) from both the CLA (31.9 ± 2.66 bpm) and the control (32.5 ± 3.87 bpm) treatment groups with regard to heart rate, 10 minutes post

exercise. The Schisandra group did not differ significantly from the combination treatment group (29.9 ± 3.16 bpm). No other significant differences were found with regards to heart rate 10 minutes post exercise.

Once again, it is clear that Schisandra has an effect on lowering heart rate values. It is possible that Schisandra increased both the oxygen supply to the muscles and carbon dioxide (and other waste products) removal from the muscles. Both these effects result in a more efficient circulatory system, and protects the heart by reducing strain as a result of overworking. This could be very beneficial in endurance racing, as a minimum heart rate has to be achieved by the horses during a race in order for them to pass the vet checks and thus continue in the race.

As with the pooled results, the addition of CLA to the Schisandra supplements (as in the combination treatment group) reduces the heart rate lowering effect of Schisandra (the combination treatment group did not differ significantly from the control group). This could be due to the fact that CLA increases metabolism, which results in an increased heart rate.

Heart rate 20 minutes post exercise

The only significant (P <0.05) difference observed between the groups was between the CLA treatment group (31.0 ± 4.27 bpm) and the Schisandra treatment group (28.2 ± 3.87 bpm). Neither of these two differed significantly from the control (32.2 ± 5.20 bpm) or combination (30.3 ± 2.46 bpm) treatment groups, and the control and combination groups did not differ significantly from each other with regard to heart rate, 20 minutes post exercise.

From these results we can see that the effects of the two supplements were: the CLA slightly decreased heart rate and the Schisandra constantly decreased heart rate. In the combination treatment group, the heart rate is lower than the control group (not significantly so), but higher than that of the Schisandra group (again, not significantly so).

Respiration rate 0 minutes post exercise

The respiration rate at 0 minutes post exercise of the CLA treatment group (26.9 ± 5.93 ipm) differed significantly (P <0.05) only from the combination treatment group (30.0 ± 5.16 ipm). The control group (24.6 ± 8.89 ipm) differed significantly from both the Schisandra group (27.6 ± 6.84 ipm), as well as the combination group. The Schisandra and combination groups did not differ significantly from each other with regard to respiration rate, 0 minutes post exercise.

The combination of the CLA and Schisandra (combination group) seems to increase the respiration rate more than each supplement would have if supplemented separately. All the groups which received a supplement (CLA, Schisandra or Combination) displayed higher respiration rates compared to the control group,

although not all of these were significant at the 95% level. This is most likely because of an increased metabolism brought on by the supplements.

Respiration rate 10 minutes post exercise

The only significant (P <0.05) difference observed in terms of respiration rate, 10 minutes post exercise, was between the CLA treatment group (25.9 ± 8.40 ipm) and the control group (20.6 ± 8.78 ipm). These two groups did not differ significantly from the Schisandra (24.0 ± 6.40 ipm) or combination (22.6 ±7.96 ipm) treatment groups. The Schisandra and combination treatment groups also did not differ significantly from each other with regards to respiration rate at 10 minutes post exercise.

The CLA significantly increased the respiration rate compared to the control group. An increased metabolism brought on by CLA would result in the increased respiration. The Schisandra group also had higher respiration rates compared to the control group, although this was not significant. It is interesting to note that the combination group had lower respiration rates than both the CLA and Schisandra groups, but slightly higher than the control group (none of these differences were significant).

Respiration rate 20 minutes post exercise

The control group (19.8 ± 7.73 ipm) differed significantly (P <0.05) from all the other treatment groups in terms of respiration rate, 20 minutes post exercise. The respiration rate of horses supplemented with CLA (25.2 ± 5.44 ipm), Schisandra (23.2 ± 8.12 ipm) and combination (22.6 ± 6.44 ipm) treatment did not differ significantly from each other at 20 minutes post exercise.

Again an increased respiration rate was observed in the groups which received supplements (CLA, Schisandra or combination).The CLA, Schisandra and combination supplements fed to the horses can be said to increase respiration rate at 0, 10 and 20 minutes after exercise (not all significantly so). It is possible that this is due to an increased metabolic rate brought on by the different supplements. It has been known for a long time that CLA stimulates the metabolism, resulting in an increased metabolic rate. S*chisandra chinensis* has been reported to stimulate respiration by increasing oxygen intake and uptake. The mechanism of this is unclear, however it is believed to be due to an increased efficiency of oxygen utilization. More studies are required to confirm these assumptions.

Post exercise parameters of the four groups of horses included in this study are summarized in Table 4.3.2.

Table 4.3.2 Least square means (LSMeans) and standard deviations (SD) of post exercise parameters for endurance horses that received dietary supplements compared to a negative control – Treatment phase results

	HR 0 min ±SD	HR 10 min ±SD	HR 20 min ±SD	Resp 0min ±SD	Resp 10min ±SD	Resp 20min ±SD
CLA (n=20)	34.7 ±4.33	31.9^{a} ±2.66	31.0^{a} ±4.27	26.9^{ac} ±5.93	25.9^{a} ±8.40	25.2^{a} ±5.44
Control (n=18)	34.9 ±3.80	32.5^{a} ±3.87	32.2^{ab} ±5.20	24.6^{a} ±8.89	20.6^{b} ±8.78	19.8^{b} ±7.73
Schisandra (n=18)	32.2 ±4.51	28.6^{b} ±2.97	28.2^{b} ±3.87	27.6^{bc} ±6.84	24.0^{ab} ±6.40	23.2^{a} ±8.12
Combination (n=16)	32.8 ±2.86	29.9^{ab} ±3.16	30.3^{ab} ±2.46	30.0^{b} ±5.16	22.6^{ab} ±7.96	22.6^{a} ±6.44

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm,) 20 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. LSMeans with different superscript letters in the same columns differed (P <0.05).

4.3.3 Haematological values

Blood pH

No significant differences were found between the four treatment groups in terms of blood pH during the treatment phase.

Erythrocyte count

The erythrocyte count of the CLA treatment group (7.10 ± 0.540 x 10^{12}/l) differed significantly (P <0.05) from that of the control (6.20 ± 0.375 x 10^{12}/l) and the Schisandra (6.41 ± 0.477 x 10^{12}/l) treatment groups, but not from the combination treatment group (6.90 ± 0.619 x 10^{12}/l). The control group differed significantly (P <0.05) from the combination treatment group, as well as the CLA treatment group, but not from the Schisandra treatment group. The Schisandra treatment group differed significantly only from the CLA treatment group with regards to erythrocyte count. The erythrocyte count of the combination treatment group differed significantly only from the control group.

The CLA group had a higher erythrocyte count than the control (this effect was significant). The CLA group had a significantly (P <0.05) higher erythrocyte count than the Schisandra group. The combination treatment

group also showed a significantly (P <0.05) higher erythrocyte count than the control group. Schisandra supplementation did not significantly increase the erythrocyte count above that of the control group. The increased erythrocyte count in the CLA and combination groups can only be as a result of CLA supplementation. It is possible that CLA stimulated splenic contractions, resulting in a release of erythrocytes into the blood stream (when used as a supplement on its own or with Schisandra). Increased erythrocyte in the blood would result in increased performance, as more oxygen would reach the muscles, increasing the muscle capacity to do aerobic work. Anaerobic respiration would also decrease if more oxygen reached the muscles, resulting in less lactic acid production.

Trendlines for these results are presented in Figure 4.3.1 (only the treatment phase is shown).

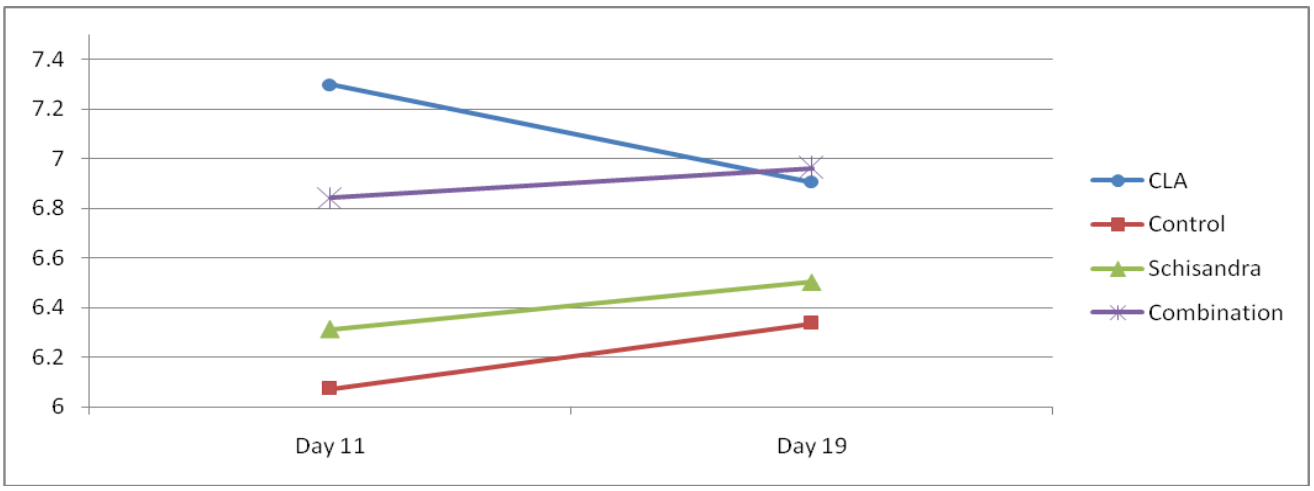

Figure 4.3.1 Changes in erythrocyte count (x 10^{12}/l) of the four treatment groups over time

Haemoglobin (Hgb)

Haemoglobin concentration of the control group (11.03 ± 0.567 g/dl) differed significantly (P <0.05) from both the CLA group (12.10 ± 0.816 g/dl) and the combination group (12.30 ± 1.094 g/dl), but not from the Schisandra group (11.28 ± 0.962 g/dl). No other significant differences between the groups were found with regards to haemoglobin concentrations.

From these results it seems possible that CLA stimulated splenic contractions, resulting in a release of erythrocytes into the blood stream (when used as a supplement on its own or with Schisandra).

Trendlines for these results are presented in Figure 4.3.2 (only the treatment phase is shown).

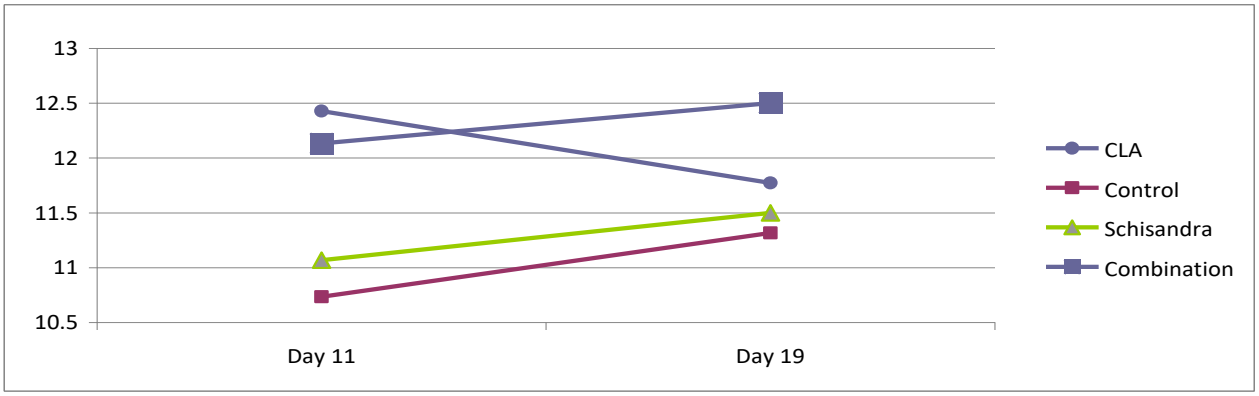

Figure 4.3.2 Changes in haemoglobin (Hgb) concentration (g/dl) of the four treatment groups over time

Leukocyte count

The only significant (P <0.05) difference observed in terms of leukocyte count was between the CLA (6.92 ± 1.748 x 10^9/l) and Schisandra (5.35 ± 0.786 x 10^9/l) treatment groups. The control (6.24 ± 0.911 x 10^9/l) and combination group (6.05 ± 1.201 x 10^9/l) did not differ significantly from each other, or any other group in terms of leukocyte count.

Many factors influence leukocyte production and concentrations: thus, these results should be interpreted with caution.

Trendlines for these results are presented in Figure 4.3.3 (only the treatment phase is shown).

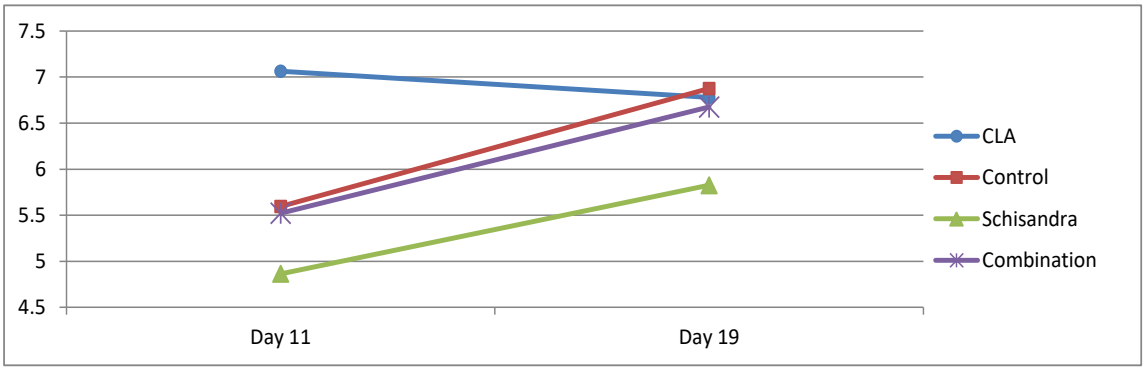

Figure 4.3.3 Changes in leukocyte count (x 10^9/l) of the four treatment groups over time

Differential cell count

No significant differences were found between the four treatment groups in terms of neutrophil, lymphocyte, monocyte, eosinophil or basophil percentages during the treatment phase.

Haematological values of the four groups of horses included in this study are summarized in Table 4.3.3.

Table 4.3.3 Least square means (LSMeans) and standard deviations (SD) of haematological values for endurance horses that received dietary supplements compared to a negative control – Treatment phase results (reference range for adult horses also indicated with reference in italics)

	Blood pH ±SD	**Ery ±SD**	**Hgb ±SD**	**Leu ±SD**	**Neu ±SD**	**Lym ±SD**	**Mon ±SD**	**Eos ±SD**	**Bas ±SD**
CLA (n=14)	7.55 ±0.038	7.10^{a} ±0.540	12.10^{a} ±0.816	6.92^{a} ±1.748	58.92 ±4.261	36.98 ±5.178	2.79 ±1.823	1.31 ±0.512	0.00 ±0.000
Control (n=12)	7.50 ±0.034	6.20^{b} ±0.375	11.03^{b} ±0.567	6.24^{ab} ±0.911	56.28 ±8.830	39.63 ±9.259	2.95 ±0.573	1.10 ±0.698	0.04 ±0.144
Schisandra (n= 12)	7.52 ±0.033	6.41^{bc} ±0.477	11.28^{ab} ±0.962	5.35^{b} ±0.786	54.28 ±4.723	41.85 ±5.569	2.59 ±1.148	1.28 ±0.915	0.00 ±0.000
Combination (n=11)	7.53 ±0.032	6.90^{ac} ±0.619	12.30^{a} ±1.094	6.05^{ab} ±1.201	55.67 ±3.867	39.98 ±3.789	2.69 ±1.106	1.33 ±0.816	0.33 ±0.730
Reference range	7.5 – 8.5 *(Hadaybit Laboratory (UAE))*	4.8 – 13 *(Hadaybit Laboratory (UAE))*	9 – 15 *(Hadaybit Laboratory (UAE))*	5 – 11 *(Hadaybit Laboratory (UAE))*	38 – 70 *(Hadaybit Laboratory (UAE))*	25 – 62 *(Hadaybit Laboratory (UAE))*	1 – 8 *(Hadaybit Laboratory (UAE))*	0.1 – 9.5 *(Hadaybit Laboratory (UAE))*	0 – 1.2 *(Hadaybit Laboratory (UAE))*
Reference range		6.0 – 10.4 *(Latimer et al., 2003)*	10 – 16 *(Latimer et al., 2003)*	5.6 - 12.1 *(Latimer et al., 2003)*	52 – 70 *(Latimer et al., 2003)*	21 – 42 *(Latimer et al., 2003)*	0 – 6 *(Latimer et al., 2003)*	0 – 7 *(Latimer et al., 2003)*	0 – 2 *(Latimer et al., 2003)*
Reference range	7.35 – 7.45 *(Davies, 2009)*	7 - 11 *(Reece, 2004)*	11.5 - 16 *(Reece, 2004)*	8 - 11 *(Reece, 2004)*	50 - 60 *(Reece, 2004)*	30 - 40 *(Reece, 2004)*	5 - 6 *(Reece, 2004)*	2 - 5 *(Reece, 2004)*	<1 *(Reece, 2004)*

Ery: Erythrocyte (x 10^{12}g/l); **Hgb:** Haemoglobin (g/dl); **Leu:** Leukocyte (x 10^{9}/l); **Neu:** Neutrophil (%); **Lym:** Lymphocyte (%); **Mon:** Monocyte (%); **Eos:** Eosinophil (%); **Bas:** Basophil (%). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.3.4 Blood metabolites

Creatinine and Lactate

No significant differences were found between the four treatment groups in terms of creatinine or lactate concentrations during the treatment phase.

The mean lactate concentrations of the CLA and Schisandra groups were below the reference range reported by Hadaybit Laboratory (UAE), Orsini *et al*. (2008) and Reece (2004). This could be due to elevated LDH concentrations (the enzyme responsible for clearing lactate from the system).

Lactate dehydrogenase (LDH)

The control group (470.67 ± 108.271 U/l) differed significantly from the CLA (596.57 ± 109.560 U/l) and combination (678.73 ± 140.958 U/l) groups, but not from the Schisandra group (528.42 ± 79.214 U/l). The CLA and combination groups did not differ significantly from each other. The Schisandra group did not differ significantly from the CLA group; however, a significant difference was found between Schisandra and the combination group.

The LDH values of the control group fell within the reference range, whereas the other three groups' LDH values were above the reference range. It is possible that both the Schisandra and CLA stimulated LDH production.

All treatment groups (CLA, Schisandra and combination supplements) had a higher enzyme concentration that the control group (although the increase in the Schisandra group was not significant). As this enzyme is responsible for clearing lactic acid (or lactate) from the body, these supplements would be very beneficial in aiding the recovery of the body after exercise.

Trendlines for these results are presented in Figure 4.3.4 (only the treatment phase is shown).

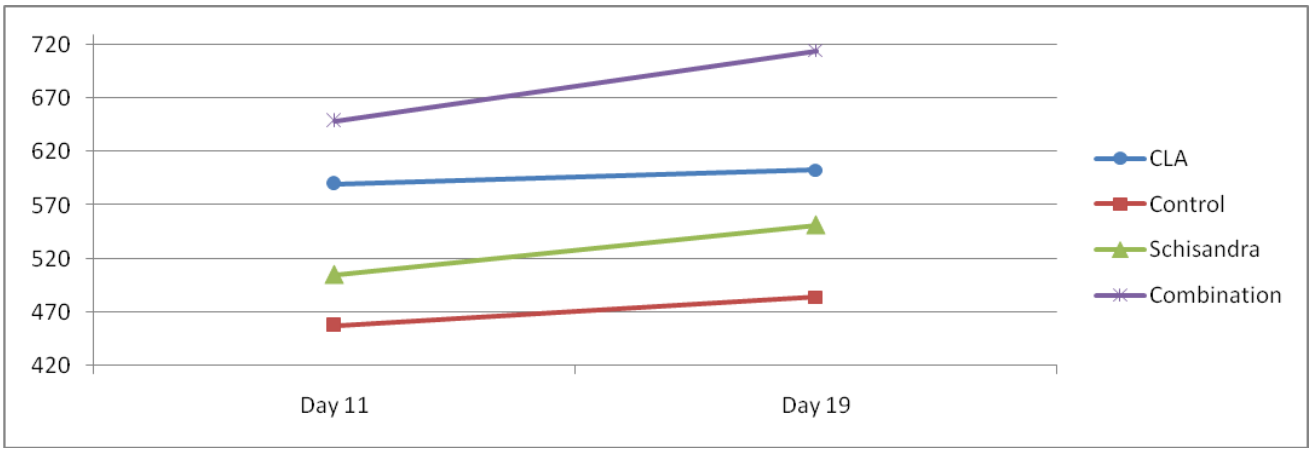

Figure 4.3.4 Changes in blood lactate dehydrogenase (LDH) concentration (U/l) of the four treatment groups over time

Blood urea nitrogen (BUN)

No significant differences were found between the four treatment groups in terms of BUN concentrations during the treatment phase.

Total cholesterol

The combination group (107.09 ± 10.977 mg/dl) displayed significantly (P <0.05) higher total cholesterol values than any other group. The mean cholesterol concentrations of the CLA (97.14 ± 7.124 mg/dl), control (91.17 ± 12.482 mg/dl) and Schisandra (94.83 ± 7.120 mg/dl) groups did not differ significantly from each other.

It seems as if the combination of CLA and Schisandra has an additive effect in increasing cholesterol concentrations in horses, as neither the CLA nor the Schisandra increased cholesterol level significantly when supplemented separately. This is possibly due to the increased amounts of energy substrates made available to the body by the different supplements.

Trendlines for these results are presented in Figure 4.3.5 (only the treatment phase is shown).

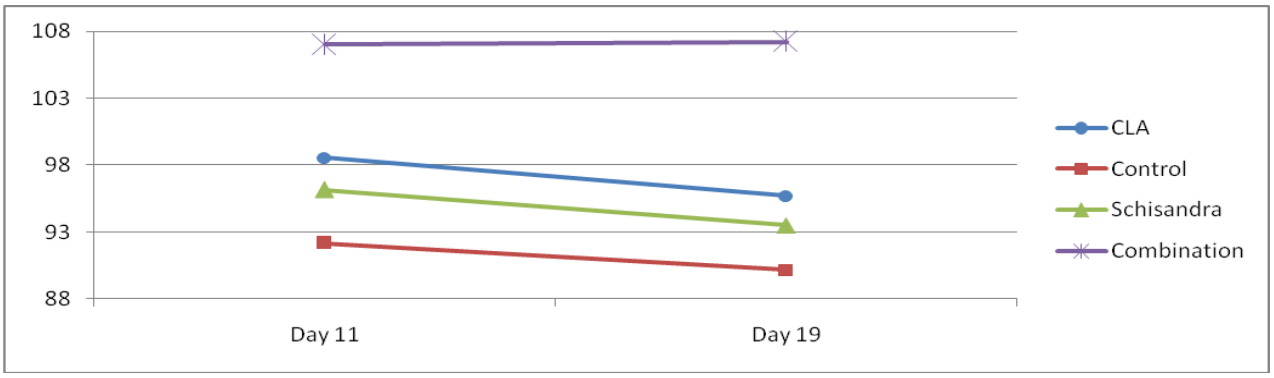

Figure 4.3.5 Changes in total cholesterol concentration (mg/dl) of the four treatment groups over time

High density lipoprotein (HDL)

Again the combination group (84.00 ± 7.239 mg/dl) displayed significantly (P <0.05) higher HDL values than any other group. The CLA (70.43 ± 6.223 mg/dl), control (68.08 ± 11.285 mg/dl) and Schisandra (67.92 ± 6.868 mg/dl) groups did not differ significantly from each other.

The large increase in total cholesterol observed in the combination group (above) was probably due to the substantial increase in HDL in the combination group.

Trendlines for these results are presented in Figure 4.3.6 (only the treatment phase is shown).

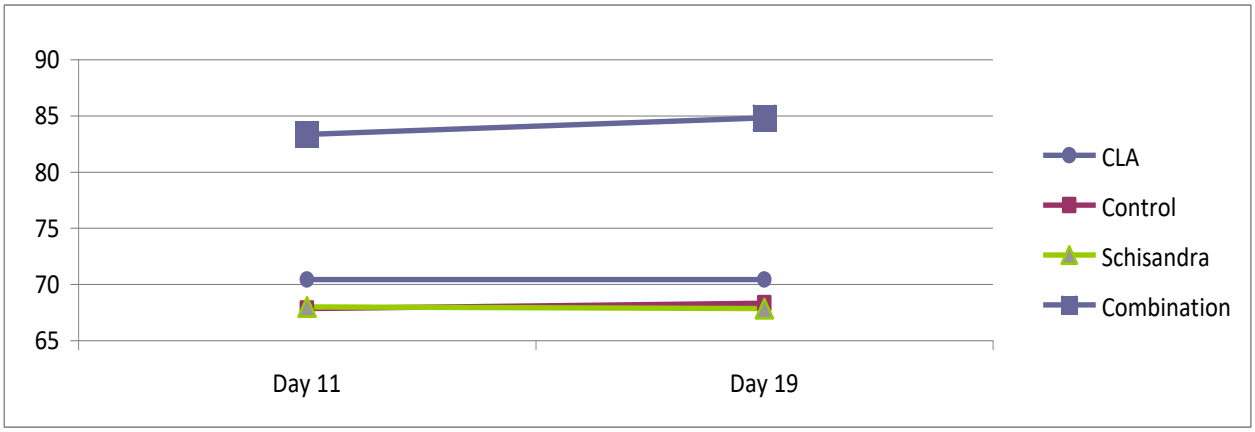

Figure 4.3.6 Changes in high density lipoprotein (HDL) concentration (mg/dl) of the four treatment groups over time

Low density lipoprotein (LDL)

No significant differences were found between the four treatment groups in terms of LDL concentrations during the treatment phase.

Blood metabolite concentrations of the four groups of horses included in this study are summarized in Table 4.3.4.

Table 4.3.4 Least square means (LSMeans) and standard deviations (SD) of blood metabolites for endurance horses that received dietary supplements compared to a negative control – Treatment phase results (reference range for adult horses also indicated with reference in italics)

	Creatinine ± SD	Lactate ± SD	LDH ± SD	BUN ± SD	Cholesterol ± SD	HDL ± SD	LDL ± SD
CLA (n=14)	1.16 ±0.096	0.76 ±0.300	596.57[ac] ±109.560	22.06 ±3.585	97.14[a] ±7.124	70.43[a] ±6.223	29.71 ±6.219
Control (n=12)	1.11 ±0.130	0.92 ±0.210	470.67[b] ±108.271	21.52 ±2.833	91.17[a] ±12.482	68.08[a] ±11.285	25.42 ±4.660
Schisandra (n=12)	1.17 ±0.209	0.89 ±0.231	528.42[ab] ±79.214	21.03 ±3.291	94.83[a] ±7.120	67.92[a] ±6.868	26.58 ±5.915
Combination (n=11)	1.14 ±0.124	0.93 ±0.213	678.73[c] ±140.958	20.36 ±3.708	107.09[b] ±10.977	84.00[b] ±7.239	26.64 ±11.690
Reference range	0.9 – 2.0 *(Hadaybit Laboratory (UAE))*	0.9 – 1.7 *(Hadaybit Laboratory (UAE))*	160 – 500 *(Hadaybit Laboratory (UAE))*	10 – 24 *(Hadaybit Laboratory (UAE))*	71 – 142 *(Hadaybit Laboratory (UAE))*	30 – 90 *(Hadaybit Laboratory (UAE))*	20 – 40 *(Hadaybit Laboratory (UAE))*
Reference range	0.4 – 2.2 *(Latimer and Prasse, 2003)*		112 – 456 *(Latimer and Prasse, 2003)*	11 – 27 *(Latimer and Prasse, 2003)*	71 – 142 *(Latimer and Prasse, 2003)*		
Reference range	0.9 – 1.9 *(Orsini et al., 2008)*	1.11 – 1.78 *(Orsini et al., 2008)*	162 – 412 *(Orsini et al., 2008)*	12 – 24 *(Orsini et al., 2008)*	75 – 150 *(Orsini et al., 2008)*		
Reference range	1 – 2 *(Reece, 2004)*	1.11 – 1.78 *(Reece, 2004)*		10 – 24 *(Reece, 2004)*	70 – 170 *(CVR Laboratory)*		

Creatinine: mg/dl; **Lactate:** mmol/l; **LDH:** Lactate dehydrogenase (U/l); **BUN:** Blood urea nitrogen (mg/dl); **Cholesterol:** mg/dl; **HDL:** High density lipoprotein (mg/dl); **LDL**: Low density lipoprotein (mg/dl)**.** (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.3.5 Blood glucose and specific hormone concentrations

Glucose, Cortisol, Triiodothyronine (T3) and Insulin

No significant differences were found between the four treatment groups in terms of glucose, cortisol, T3 or insulin concentrations during the treatment phase.

Thyroxin (T4)

T4 concentration of the combination (28.66 ± 6.667 nmol/l) group differed significantly (P <0.05) from both the control (37.77 ± 9.333 nmol/l) and the Schisandra (37.77 ± 7.331 nmol/l) treatment groups; yet, the control and Schisandra treatment groups did not differ significantly from each other. The CLA group (32.28 ± 7.000 nmol/l) did not differ significantly from any other group in terms of T4 concentration.

It seems as if CLA has an effect on lowering blood T4 concentrations, especially when supplemented in combination with Schisandra. It could be that the combination of CLA and Schisandra stimulates the conversion of T4 (physiologically less active) to T3 (physiologically more active), in order to increase metabolic rate.

Trendlines for these results are presented in Figure 4.3.7 (only the treatment phase is shown).

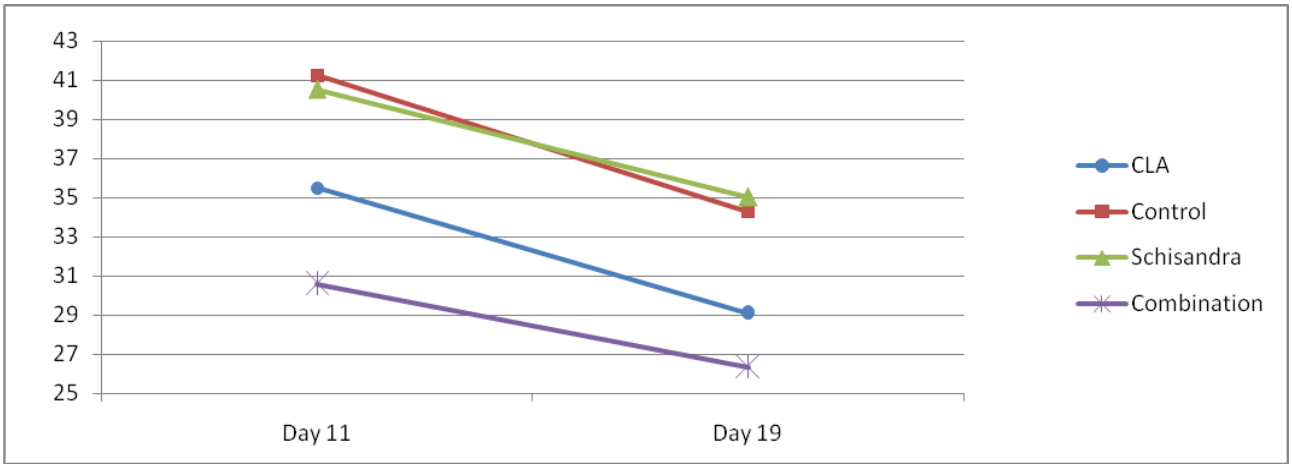

Figure 4.3.7 Changes in blood T4 concentration (nmol/l) of the four treatment groups over time

Blood glucose and specific hormone concentrations of the four groups of horses included in this study are summarized in Table 4.3.5.

Table 4.3.5 Least square means (LSMeans) and standard deviations (SD) of blood glucose and specific hormone concentrations for endurance horses that received dietary supplements compared to a negative control – Treatment phase results (reference range for adult horses also indicated with reference in italics)

	Glucose ±SD	**Cortisol ±SD**	**T3 ±SD**	**T4 ±SD**	**Insulin ±SD**
CLA (n=14)	104.93 ±6.719	97.96 ±24.957	0.74 ±0.223	32.28^{ab} ±7.000	5.36 ±3.317
Control (n=12)	119.25 ±23.336	98.29 ±24.211	0.67 ±0.253	37.77^{a} ±9.333	7.19 ±6.326
Schisandra (n=12)	120.83 ±21.788	97.20 ±36.556	0.64 ±0.170	37.77^{a} ±7.331	7.55 ±6.946
Combination (n=11)	109.90 ±12.112	93.09 ±31.437	0.63 ±0.104	28.66^{b} ±6.667	4.99 ±4.546
Reference range	75 – 115 *(Orsini et al., 2008)*				10 – 30 *(Orsini et al., 2008)*
Reference range		69 – 180 *(CVR Laboratory)*	0.24 – 1.28 *(CVR Laboratory)*	16.5 – 24.4 *(CVR Laboratory)*	5.7 – 31.4 *(CVR Laboratory)*
Reference range	62 – 114 *(Hadaybit Laboratory (UAE))*	90 – 200 *(Idexx Laboratory (RSA))*	0.5 – 2.5 *(Idexx Laboratory (RSA))*	11 – 37 *(Idexx Laboratory (RSA))*	4 – 20 *(Idexx Laboratory (RSA))*
Reference range	62 – 134 *(Latimer and Prasse, 2003)*		0.3 – 2.9 *(Robinson & Sprayberry, 2009)*	6 – 46 *(Robinson & Sprayberry, 2009)*	

Glucose: mg/dl; **Cortisol:** nmol/l; **T3:** Triiodothyronine (nmol/l); **T4:** Thyroxin (nmol/l); **Insulin:** (µlU/l). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4. Results for specific days:

Day 0: Start of adaptation phase

These measurements were recorded in order to evaluate the homogeneity of the groups of horses used at the start of the trial period.

4.4.1 Pre-exercise basal physiological parameters on Day 0

Basal heart rate

The only significant (P <0.05) difference in basal heart rate (beats per minute/bpm) on day 0 was between the control group (35.0 ± 4.82 bpm) and the Schisandra treatment group (27.2 ± 3.49 bpm). The CLA (33.1 ± 2.04 bpm) and combination (29.7 ± 6.77 bpm) treatment groups did not differ significantly from each other or the above mentioned two groups in terms of basal heart rate on day 0.

As no supplements had been given to the horses yet, this effect is probably due to differences in fitness between the groups of horses.

Basal respiration rate and rectal temperature

No significant differences were found between the four treatment groups in terms of basal respiration rate (inhalations per minute/ipm) or rectal temperature on day 0.

The basal temperature of all four groups was lower than the reference range reported by Huntington *et al*. (2004) and Robertshaw (2004). This could be due to cool weather as the trial took place during the winter months, and because the temperature recordings were done early in the mornings (around 5am).

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 4.4.1

Table 4.4.1 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control on day 0 (reference range for adult horses also indicated with reference in italics)

	Basal HR ± SD	**Basal Resp ± SD**	**Basal Temp ± SD**
CLA (n=7)	33.1[ab] ±2.04	13.0 ±1.63	36.5 ±0.28
Control (n=6)	35.0 [a] ±4.82	12.8 ±1.83	36.6 ±0.46
Schisandra (n=6)	27.2 [b] ±3.49	13.5 ±2.17	36.3 ±0.64
Combination (n=6)	29.7 [ab] ±6.77	13.5 ±1.76	36.7 ±0.36
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington et al., 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.2 Post exercise parameters on Day 0

Heart rate values

No significant differences were found between the four treatment groups in terms of heart rate at 0, 10 and 20 minutes post exercise on day 0.

Trendlines for these results are presented in Figure 4.4.1.

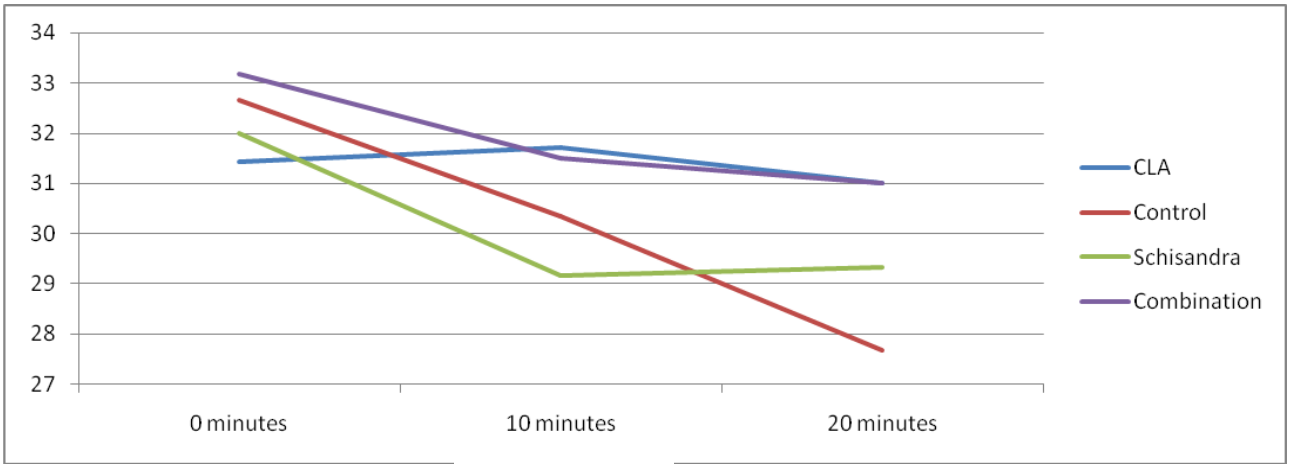

Figure 4.4.1. Changes in heart rate (beats per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 0

Respiration rate 0 minutes post exercise

Respiration rate, 0 minutes post exercise, of the CLA treatment group (20.6 ± 4.76 ipm) differed significantly (P <0.05) from the combination treatment group (34.0 ± 4.90 ipm), but not from the control group (15.3 ± 4.374 ipm) or the Schisandra treatment group (27.7 ± 9.83 ipm) on day 0. The control group differed significantly (P <0.05) from the combination treatment group as well as the Schisandra treatment group in terms of respiration rate, 0 minutes post exercise on day 0. The respiration rate of the combination treatment group did not differ significantly from the Schisandra treatment group directly after exercise on day 0. This is probably due to differences in fitness levels between the horses; the control group had the fittest horses, then the CLA group, then the Schisandra group and the least fit was the combination group (on Day 0).

Respiration rate 10 minutes post exercise

The only significant (P <0.05) difference in respiration rate at 10 minutes post exercise on day 0 was found between the control group (14.2 ± 3.71 ipm) and the combination (25.3 ± 7.45 ipm) group. The CLA (20.7 ± 1.11 ipm) and Schisandra (22.0 ± 6.57 ipm) groups did not differ significantly from each other or the above two groups in terms of respiration rate, 10 minutes post exercise on day 0.

This is again probably due to differences in fitness levels between the groups of horses.

Respiration rate 20 minutes post exercise

The CLA treatment group (22.9 ± 3.76 ipm) differed significantly (P <0.05) from the control group (14.0 ± 3.95 ipm) in terms of respiration rate 20 minutes post exercise on day 0. The respiration rate of Schisandra

(19.3 ± 7.34 ipm) and combination (22.0 ± 5.51 ipm) treatment groups did not differ significantly from each other or any other group at 20 minutes post exercise on day 0.

Trendlines for these results are presented in Figure 4.4.2.

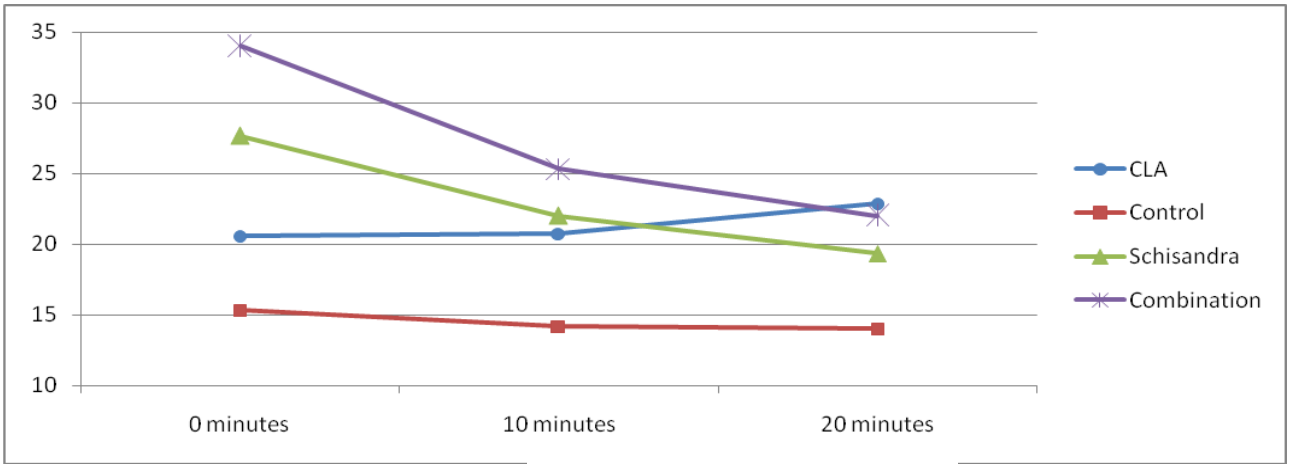

Figure 4.4.2. Changes in respiration rate (number of inhalations per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 0

Post exercise parameters of the four groups of horses included in this study are summarized in Table 4.4.2.

Table 4.4.2 Least square means (LSMeans) and standard deviations (SD) of post exercise parameters for endurance horses that received dietary supplements compared to a negative control on day 0

	HR 0 min ± SD	HR 10 min ± SD	HR 20 min ± SD	Resp 0 min ± SD	Resp 10 min ± SD	Resp 20 min ± SD
CLA (n=7)	31.4 ±3.95	31.7 ±5.22	31.0 ±4.65	20.6^{ac} ±4.76	20.7 ^{ab} ±1.11	22.9^{a} ±3.76
Control (n=6)	32.7 ±4.13	30.3 ±8.57	27.7 ±4.72	15.3^{c} ±4.374	14.2 ^{a} ±3.71	14.0^{b} ±3.95
Schisandra (n=6)	32.0 ±8.79	29.2 ±8.26	29.3 ±8.80	27.7^{ab} ±9.83	22.0 ^{ab} ±6.57	19.3^{ab} ±7.34
Combination (n=6)	33.2 ±3.76	31.5 ±3.02	31.0 ±2.97	34.0^{b} ±4.90	25.3 ^{b} ±7.45	22.0^{ab} ±5.51

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm) 20 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.3 Haematological values on Day 0

Blood pH, erythrocyte count, haemoglobin (Hgb) and leukocyte count

No significant differences were found between the four treatment groups in terms of blood pH, erythrocyte count, Hgb and leukocyte count on day 0.

The haemoglobin concentration of the CLA group (12.26 ± 0.611 g/dl) was slightly higher than that of the reference range reported by Hadaybit Laboratory (UAE), Latimer *et al*. (2003) and Reece (2004); however, it was not significantly higher than any of the other three treatment groups.

Differential cell count

No significant differences were found between the four treatment groups in terms of neutrophil, lymphocyte, monocyte, eosinophil and basophil percentages on day 0.

As there were no significant differences for haematological values between the four groups, it can be concluded that the horses were in the same physiological state, and that the four groups of horses were homogenous.

Haematological values of the four groups of horses included in this study are summarized in Table 4.4.3.

Table 4.4.3 Least square means (LSMeans) and standard deviations (SD) of haematological values for endurance horses that received dietary supplements compared to a negative control on day 0 (reference range for adult horses also indicated with reference in italics)

	Blood pH ±SD	**Ery ±SD**	**Hgb ±SD**	**Leu ±SD**	**Neu ±SD**	**Lym ±SD**	**Mon ±SD**	**Eos ±SD**	**Bas ±SD**
CLA (n=7)	7.55 ±0.041	7.16 ±0.550	12.26 ±0.611	6.65 ±1.142	57.00 ±4.995	38.71 ±6.317	3.07 ±2.232	1.21 ±0.389	0.00 ±0.000
Control (n=6)	7.53 ±0.033	6.54 ±0.634	11.55 ±0.981	6.51 ±1.057	58.10 ±7.145	39.12 ±7.642	1.70 ±0.610	1.08 ±0.458	0.00 ±0.000
Schisandra (n=6)	7.52 ±0.158	6.69 ±0.363	11.73 ±0.907	5.38 ±0.457	57.48 ±4.843	38.90 ±4.883	2.07 ±1.035	0.90 ±0.358	0.65 ±1.592
Combination (n=6)	7.55 ±0.042	6.60 ±0.676	11.72 ±1.100	6.42 ±0.989	52.85 ±5.494	43.12 ±5.883	2.60 ±1.293	1.43 ±0.706	0.00 ±0.000
Reference range	7.5 – 8.5 *(Hadaybit Laboratory (UAE))*	4.8 – 13 *(Hadaybit Laboratory (UAE))*	9 – 15 *(Hadaybit Laboratory (UAE))*	5 – 11 *(Hadaybit Laboratory (UAE))*	38 – 70 *(Hadaybit Laboratory (UAE))*	25 – 62 *(Hadaybit Laboratory (UAE))*	1 – 8 *(Hadaybit Laboratory (UAE))*	0.1 – 9.5 *(Hadaybit Laboratory (UAE))*	0 – 1.2 *(Hadaybit Laboratory (UAE))*
Reference range		6.0 – 10.4 *(Latimer et al., 2003)*	10 – 16 *(Latimer et al., 2003)*	5.6 - 12.1 *(Latimer et al., 2003)*	52 – 70 *(Latimer et al., 2003)*	21 – 42 *(Latimer et al., 2003)*	0 – 6 *(Latimer et al., 2003)*	0 – 7 *(Latimer et al., 2003)*	0 – 2 *(Latimer et al., 2003)*
Reference range	7.35 – 7.45 *(Davies, 2009)*	7 - 11 *(Reece, 2004)*	11.5 - 16 *(Reece, 2004)*	8 - 11 *(Reece, 2004)*	50 - 60 *(Reece, 2004)*	30 - 40 *(Reece, 2004)*	5 - 6 *(Reece, 2004)*	2 - 5 *(Reece, 2004)*	<1 *(Reece, 2004)*

Ery: Erythrocyte (x 10^{12}g/l); **Hgb:** Haemoglobin (g/dl); **Leu:** Leukocyte (x 10^{9}/l); **Neu:** Neutrophil (%); **Lym:** Lymphocyte (%); **Mon:** Monocyte (%); **Eos:** Eosinophil (%); **Bas:** Basophil (%). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.4 Blood metabolites on Day 0

Creatinine and lactate

No significant differences were found between the four treatment groups in terms of creatinine or lactate concentration on day 0.

The lactate concentrations of all four treatment groups were slightly below that of the reference range reported by Hadaybit Laboratory (UAE), Orsini *et al*. (2008) and Reece (2004). This is probably because the horses were fit and had elevated LDH concentrations which led to the horses being able to metabolize lactate more effectively.

Lactate dehydrogenase (LDH)

No significant differences were found between the four treatment groups in terms of LDH concentration on day 0. The LDH of the CLA, Schisandra and combination treatment groups were higher than that of the reference range reported by Hadaybit Laboratory (UAE), Latimer and Prasse (2003) and Orsini *et al*. (2008). As these were fit, hard working horses at the end of a racing season, they were able to effectively break down lactate in order to recover from strenuous exercise. It can thus be expected that the LDH values will be above the reference range for average horses (not adapted to this intensity of exercise).

Blood urea nitrogen (BUN)

No significant differences were found between the four treatment groups in terms of BUN concentration on day 0. The BUN concentrations of all four groups were slightly higher than the reference range reported by Hadaybit Laboratory (UAE), Latimer and Prasse (2003), Orsini *et al*. (2008) and Reece (2004). This could be due to a high protein turnover of the horses' muscles as a result of high exercise intensities before start of trial.

Lipogram

No significant differences were found between the four treatment groups in terms of cholesterol, HDL or LDL concentration on day 0.

As there were no significant differences for blood metabolites between the four groups, it can be concluded that the horses were in the same physiological state, and that the four groups of horses were homogenous.

Blood metabolite concentrations of the four groups of horses included in this study are summarized in Table 4.4.4.

Table 4.4.4 Least square means (LSMeans) and standard deviations (SD) of blood metabolites for endurance horses that received dietary supplements compared to a negative control on day 0 (reference range for adult horses also indicated with reference in italics)

	Creatinine ± SD	**Lactate ± SD**	**LDH ± SD**	**BUN ± SD**	**Cholesterol ±SD**	**HDL ±SD**	**LDL ±SD**
CLA (n=7)	1.05 ±0.081	0.75 ±0.099	648.71 ±199.436	29.71 ±0.997	95.71 ±7.994	70.14 ±6.768	29.43 ±8.923
Control (n=6)	0.99 ±0.096	0.75 ±0.181	474.00 ±101.147	29.38 ±2.197	92.00 ±13.784	67.67 ±11.290	26.33 ±7.090
Schisandra (n=6)	1.08 ±0.179	0.69 ±0.094	505.33 ±38.203	28.73 ±1.766	98.67 ±6.890	71.83 ±8.085	30.50 ±4.135
Combination (n=6)	0.98 ±0.066	0.86 ±0.127	661.67 ±114.612	27.10 ±0.642	104.00 ±10.373	81.33 ±6.314	25.67 ±8.524
Reference range	0.9 – 2.0 *(Hadaybit Laboratory (UAE))*	0.9 – 1.7 *(Hadaybit Laboratory (UAE))*	160 – 500 *(Hadaybit Laboratory (UAE))*	10 – 24 *(Hadaybit Laboratory (UAE))*	71 – 142 *(Hadaybit Laboratory (UAE))*	30 – 90 *(Hadaybit Laboratory (UAE))*	20 – 40 *(Hadaybit Laboratory (UAE))*
Reference range	0.4 – 2.2 *(Latimer and Prasse, 2003)*		112 – 456 *(Latimer and Prasse, 2003)*	11 – 27 *(Latimer and Prasse, 2003)*	71 – 142 *(Latimer and Prasse, 2003)*		
Reference range	0.9 – 1.9 *(Orsini et al., 2008)*	1.11 – 1.78 *(Orsini et al., 2008)*	162 – 412 *(Orsini et al., 2008)*	12 – 24 *(Orsini et al., 2008)*	75 – 150 *(Orsini et al., 2008)*		
Reference range	1 – 2 *(Reece, 2004)*	1.11 – 1.78 *(Reece, 2004)*		10 – 24 *(Reece, 2004)*	70 – 170 *(CVR Laboratory)*		

Creatinine: mg/dl; **Lactate:** mmol/l; **LDH:** Lactate dehydrogenase (U/l); **BUN:** Blood urea nitrogen (mg/dl); **Cholesterol:** mg/dl; **HDL:** High density lipoprotein (mg/dl); **LDL**: Low density lipoprotein (mg/dl)**.** (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.5 Blood glucose and specific hormone concentrations on Day 0

Glucose, cortisol, T3, T4 and insulin

No significant differences were found between the four treatment groups in terms of glucose, cortisol, T3, T4 or insulin concentration on day 0.

The insulin concentrations of the control and Schisandra groups were slightly below the reference range reported by Orsini *et al*. (2008), CVR Laboratory and Idexx Laboratory (RSA).

As there were no significant differences for blood glucose and specific hormones between the four groups, it can be concluded that the horses were in the same physiological state, and that the four groups of horses were homogenous.

Blood glucose and specific hormone concentrations of the four groups of horses included in this study are summarized in Table 4.4.5.

Table 4.4.5 Least square means (LSMeans) and standard deviations (SD) of blood glucose and specific hormone concentrations for endurance horses that received dietary supplements compared to a negative control on day 0 (reference range for adult horses also indicated with reference in italics)

	Glucose ±SD	**Cortisol ±SD**	**T3 ±SD**	**T4 ±SD**	**Insulin ±SD**
CLA (n=7)	107.43 ±15.736	99.51 ±15.078	0.57 ±0.052	25.68 ±5.358	6.45 ±4.147
Control (n=6)	110.67 ±5.680	110.02 ±36.645	0.72 ±0.426	33.78 ±7.924	3.85 ±4.532
Schisandra (n=6)	104.33 ±6.055	115.40 ±18.013	0.54 ±0.000	33.22 ±8.111	2.24 ±0.480
Combination (n=6)	114.17 ±10.759	123.50 ±20.744	0.57 ±0.079	25.21 ±7.213	4.73 ±4.795
Reference range	75 – 115 *(Orsini et al., 2008)*				10 – 30 *(Orsini et al., 2008)*
Reference range		69 – 180 *(CVR Laboratory)*	0.24 – 1.28 *(CVR Laboratory)*	16.5 – 24.4 *(CVR Laboratory)*	5.7 – 31.4 *(CVR Laboratory)*
Reference range	62 – 114 *(Hadaybit Laboratory (UAE))*	90 – 200 *(Idexx Laboratory (RSA))*	0.5 – 2.5 *(Idexx Laboratory (RSA))*	11 – 37 *(Idexx Laboratory (RSA))*	4 – 20 *(Idexx Laboratory (RSA))*
Reference range	62 – 134 *(Latimer and Prasse, 2003)*		0.3 – 2.9 *(Robinson & Sprayberry, 2009)*	6 – 46 *(Robinson & Sprayberry, 2009)*	

Glucose: mg/dl; **Cortisol:** nmol/l; **T3:** Triiodothyronine (nmol/l); **T4:** Thyroxin (nmol/l); **Insulin:** (µlU/l). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

Day 5: Adaptation phase

The uniformity of the four groups of horses was evaluated after participating in the study for five days. After five days on treatment horses were exposed to the same environment, treatment and exercise (adaptation phase).

4.4.6 Pre-exercise basal physiological parameters on Day 5

Basal heart and respiration rate

No significant differences were found between the four treatment groups in terms of basal heart rate (beats per minute/bpm) or respiration rates (inhalations per minute/ipm) on day 5.

Since the basal heart rates and respiration rates did not differ statistically, it can be concluded that the horses had reached the same fitness levels by day 5.

Basal temperature (rectal)

No significant differences were found between the four treatment groups in terms of basal rectal temperatures on day 5. All four groups' rectal temperatures were slightly below the reference range reported by Huntington *et al*. (2004) and Robertshaw (2004). This could be because of cool winter climates and early morning temperature recordings.

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 7.4.6.

Table 4.4.6 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control on day 5 (reference range for adult horses also indicated with reference in italics)

	Basal HR ± SD	**Basal Resp ± SD**	**Basal Temp ± SD**
CLA (n=7)	29.4 ±5.74	11.7 ±0.76	36.0 ±0.64
Control (n=6)	31.3 ±3.83	10.7 ±2.07	36.8 ±0.35
Schisandra (n=6)	24.8 ±2.64	11.0 ±2.10	36.5 ±0.59
Combination (n=6)	30.2 ±4.71	9.3 ±1.03	36.0 ±0.53
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington et al., 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.7 Post exercise parameters on Day 5

Heart rates post exercise

No significant differences were found between the four treatment groups in terms of heart rate at 0, 10 or 20 minutes post exercise on day 5.

Trendlines for these results are presented in Figure 4.4.3.

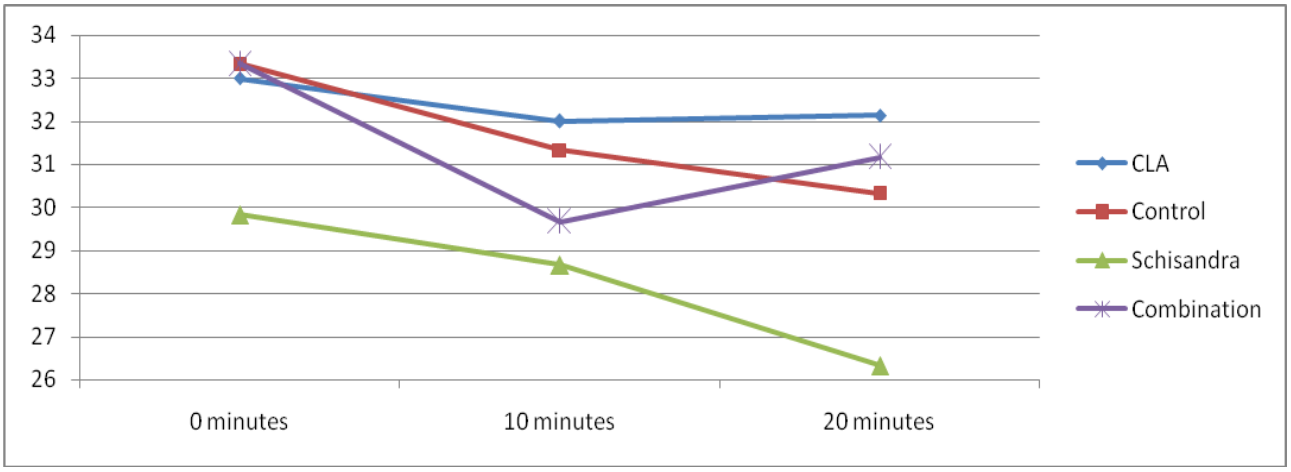

Figure 4.4.3.Changes in heart rate (beats per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 5

Respiration rates post exercise

No significant differences were found between the four treatment groups in terms of respiration rate at 0, 10 or 20 minutes post exercise on day 5.

The post-exercise heart rates and respiration rates did not differ significantly between the four treatment groups on day 5. From this it can be concluded that the horses all reached the same fitness levels by day 5.

Trendlines for these results are presented in Figure 4.4.4.

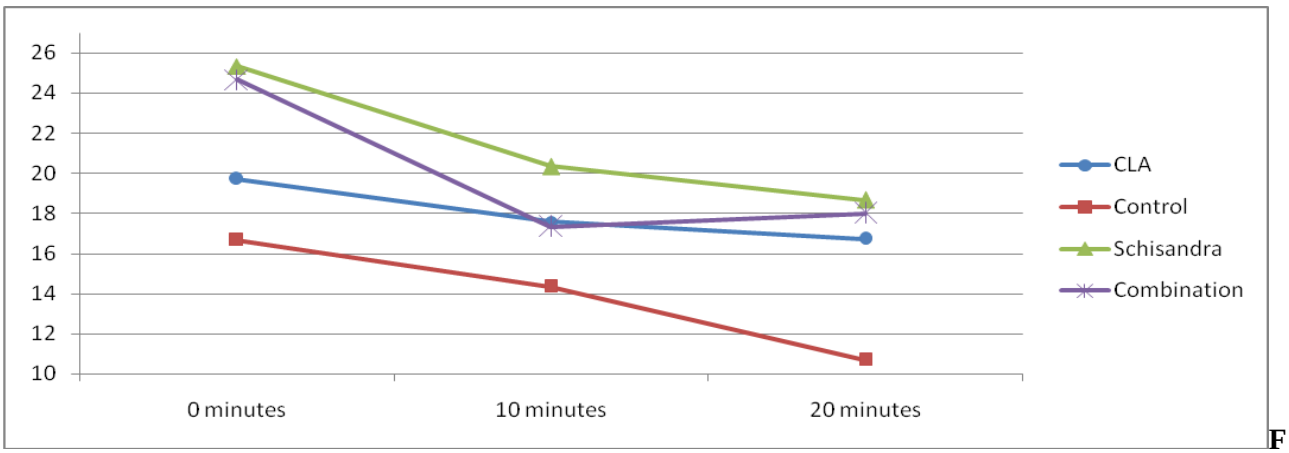

Figure 4.4.4 Changes in respiration rate (inhalations per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 5

Post-exercise parameters of the four groups of horses included in this study are summarized in Table 4.4.7.

Table 4.4.7 Least square means (LSMeans) and standard deviations (SD) of post exercise parameters for endurance horses that received dietary supplements compared to a negative control on day 5

	HR 0 min ± SD	HR 10 min ± SD	HR 20 min ± SD	Resp 0 min ± SD	Resp 10 min ± SD	Resp 20 min ± SD
CLA (n=7)	33.0 ±3.79	32.0 ±3.21	32.1 ±4.67	19.7 ±6.87	17.6 ±3.82	16.7 ±2.36
Control (n=6)	33.3 ±3.08	31.3 ±4.63	30.3 ±2.94	16.7 ±6.89	14.3 ±8.89	10.7 ±4.68
Schisandra (n=6)	29.8 ±3.60	28.7 ±1.97	26.3 ±3.98	25.3 ±12.44	20.3 ±10.69	18.7 ±7.12
Combination (n=6)	33.3 ±5.57	29.7 ±4.93	31.2 ±3.49	24.7 ±7.97	17.3 ±5.75	18.0 ±5.51

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm) 20 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. LSMeans with different superscript letters in the same columns differed (P <0.05).

Day 10: Start of supplementation

These measurements were recorded in order to determine whether a single dose of supplements had an immediate effect on heart rates and respiration rates (basal and post exercise).

4.4.8 Pre-exercise basal physiological parameters on Day 10

Basal heart and respiration rate

No significant differences were found between the four treatment groups in terms of basal heart rate (beats per minute/bpm) or respiration rate (inhalations per minute/ipm) on day 10.

Since the basal heart rates and respiration rates did not differ statistically, it can be concluded that the groups of horses were uniform in terms of fitness levels.

Basal temperature (rectal)

No significant differences were found between the four treatment groups in terms of basal rectal temperatures on day 10. All four groups' rectal temperatures were slightly below the reference range

reported by Huntington *et al*. (2004) and Robertshaw (2004). This could be due to cool winter climates and early morning temperature recordings.

As these measurements were recorded directly after the horses' morning feed, it is improbable that enough time had passed for the supplements to have been absorbed into the blood stream, in order for them to have their effects on basal values.

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 4.4.8.

Table 4.4.8 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control on day 10 (reference range for adult horses also indicated with reference in italics)

	Basal HR ± SD	**Basal Resp ± SD**	**Basal Temp ± SD**
CLA (n=7)	29.2 ±5.00	14.0 ±3.79	36.9 ±0.48
Control (n=6)	29.2 ±4.26	10.7 ±2.42	36.8 ±0.19
Schisandra (n=6)	28.2 ±4.62	12.7 ±4.50	36.4 ±0.47
Combination (n=6)	26.8 ±2.77	10.0 ±1.41	36.6 ±0.40
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington et al., 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.9 Post exercise parameters on Day 10

Heart rates post exercise

No significant differences were found between the four treatment groups in terms of heart rate at 0, 10 or 20 minutes post exercise on day 10. It is possible that the supplements were not yet fully absorbed by the horses for their effects to be noted.

Trendlines for these results are presented in Figure 4.4.5.

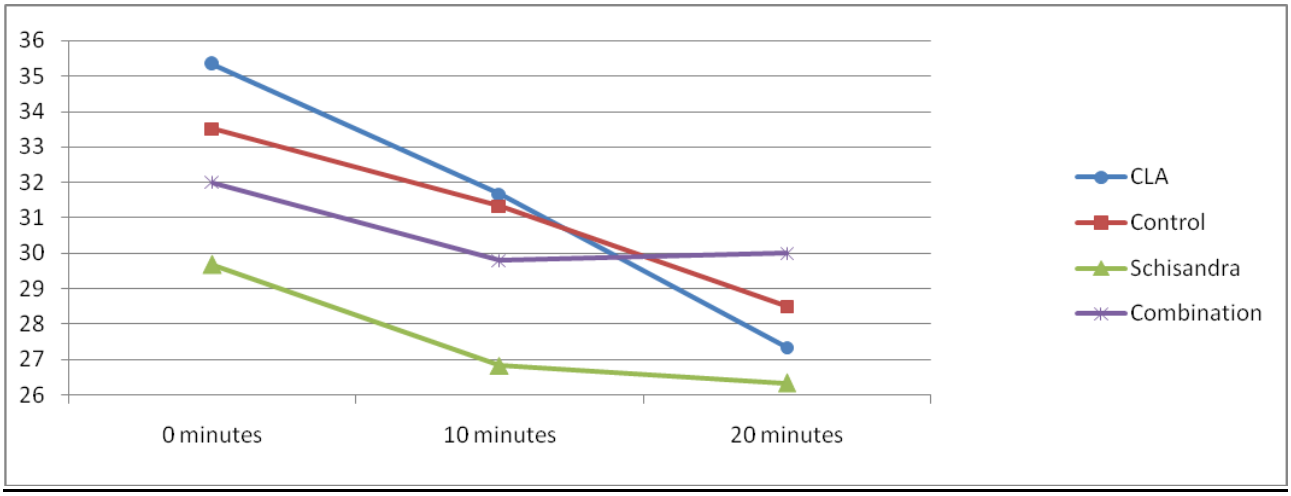

Figure 4.4.5 Changes in heart rate (beats per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 10

Respiration rates 0 and 10 minutes post exercise

No significant differences were found between the four treatment groups in terms of respiration rate at 0 or 10 minutes post exercise on day 10.

Respiration rate 20 minutes post exercise

Both the CLA (21.0 ± 3.29 ipm) and combination (19.2 ± 3.03 ipm) treatment groups differed significantly (P <0.05) from the control group (12.3 ± 2.34 ipm), but not from each other, in terms of respiration rate 20 minute post exercise on day 10. The Schisandra treatment group (18.3 ± 5.43 ipm) did not differ significantly from any other group with regards to respiration rate, 20 minute post exercise on day 10.

The CLA had an effect in increasing respiration rate after exercise (when supplemented separately or in combination with Schisandra). This could be a result of a higher metabolism brought on by CLA supplementation.

Trendlines for these results are presented in Figure 4.4.6.

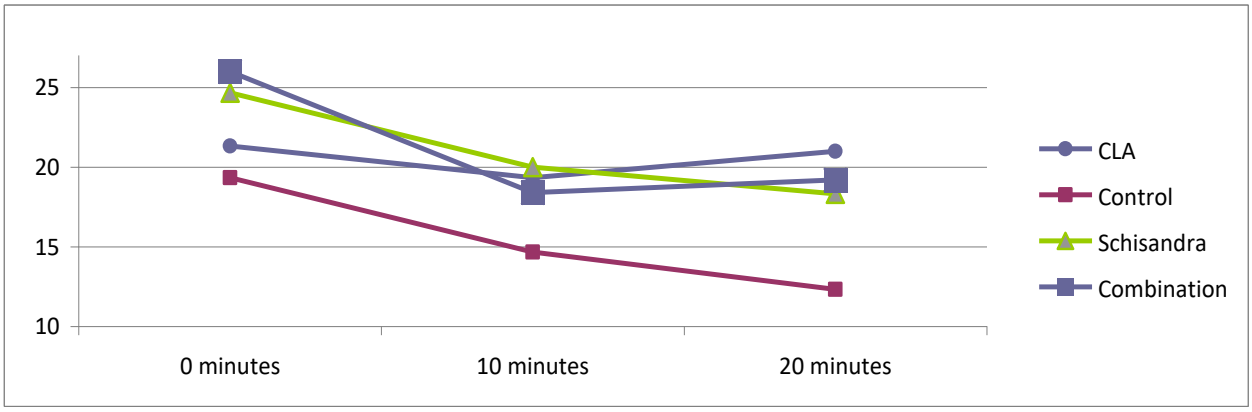

Figure 4.4.6 Changes in respiration rate (inhalations per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 10.

Post exercise parameters of the four groups of horses included in this study are summarized in Table 4.4.9.

Table 4.4.9 Least square means (LSMeans) and standard deviations (SD) of post exercise parameters for endurance horses that received dietary supplements compared to a negative control on day 10

	HR 0 min ± SD	HR 10 min ± SD	HR 20 min ± SD	Resp 0 min ± SD	Resp 10 min ± SD	Resp 20 min ± SD
CLA (n=6)	35.3 ±6.35	31.7 ±2.58	27.3 ±4.50	21.3 ±3.01	19.3 ±3.93	21.0[a] ±3.29
Control (n=6)	33.5 ±3.02	31.3 ±3.67	28.5 ±4.64	19.3 ±6.53	14.7 ±4.13	12.3[b] ±2.34
Schisandra (n=6)	29.7 ±4.13	26.8 ±3.76	26.3 ±2.80	24.7 ±5.47	20.0 ±5.66	18.3[ab] ±5.43
Combination (n=6)	32.0 ±3.39	29.8 ±3.49	30.0 ±2.35	26.0 ±2.00	18.4 ±3.29	19.2[a] ±3.03

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm) 20 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. LSMeans with different superscript letters in the same columns differed (P <0.05).

Day 11: Supplementation

In order to evaluate the short-term effects (as the horses received only two supplement doses: day 10 and day 11) of supplements on horses, their effects on basal heart and respiration rates, as well the effects on haematological parameters, energy provision and certain hormones, were determined.

4.4.10 Pre-exercise basal physiological parameters on Day 11

Basal heart rate, respiration rate and rectal temperature

No significant differences were found between the four treatment groups in terms of basal heart rate (beats per minute/bpm), respiration rate (inhalations per minute/ipm) or basal rectal temperature on day 11.

All four groups' rectal temperatures were slightly below the reference range reported by Huntington *et al*. (2004) and Robertshaw (2004), which might be due to the cool winter climates and early morning temperature recordings.

Since the basal heart rates and respiration rates did not differ statistically, it can be concluded that the groups of horses were uniform in terms of fitness levels on day 11.

It may be that more than one dose of the supplements is required to significantly alter basal heart rate, respiration and temperature values.

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 4.4.10.

Table 4.4.10 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control on day 11 (reference range for adult horses also indicated with reference in italics)

	Basal HR ± SD	**Basal Resp ± SD**	**Basal Temp ± SD**
CLA (n=7)	31.9 ±4.67	12.0 ±3.06	36.5 ±0.46
Control (n=6)	31.2 ±4.75	9.3 ±1.63	36.8 ±0.77
Schisandra (n=6)	25.7 ±2.34	10.7 ±3.50	36.5 ±0.50
Combination (n=6)	28.5 ±4.51	11.0 ±3.29	36.4 ±0.22
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington et al., 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.11 Haematological values on Day 11

Blood pH

Blood pH of the CLA group (7.57 ± 0.034) differed significantly (P <0.05) from both the control (7.49 ± 0.021) and Schisandra (7.51 ± 0.032) groups; although, the control and Schisandra groups did not differ significantly from each other. Blood pH of the combination (7.54 ± 0.039) group did not differ significantly from any other treatment group.

When supplemented separately, the CLA and Schisandra marginally increased the blood pH above that of the control group. The increase is very small (0.0797 and 0.0216, respectively) and thus neglible. This is a once-off effect, as no significant differences were observed between the groups in the pooled and treatment phase results. There were also no significant differences in blood pH between the groups on day 19.

Erythrocyte count

The erythrocyte count of the CLA treatment group (7.30 ± 0.343 x 10^{12}/l) differed significantly (P <0.05) from both the control (6.07 ± 0.397 x 10^{12}/l) and Schisandra group (6.31 ± 0.510 x 10^{12}/l) on day 11. However, the erythrocyte count of the control and Schisandra group did not differ significantly from each other. Although the combination treatment group (6.84 ± 0.693 x 10^{12}/l) did not differ significantly from any other group in terms of erythrocyte count, it did have a slightly higher erythrocyte value than the control group.

From these results it seems possible that CLA stimulated splenic contractions, resulting in a release of erythrocytes into the blood stream (when used as a supplement on its own or with Schisandra). An increased erythrocyte count in the blood would result in increased performance, as more oxygen would reach the muscles, increasing the muscle capacity to do aerobic work. Anaerobic respiration would also decrease if more oxygen reached the muscles, resulting in less lactic acid production.

Haemoglobin (Hgb)

Haemoglobin concentration of the control group (10.73 ± 0.582 g/dl) differed significantly (P <0.05) from the CLA group (12.43 ± 0.522 g/dl) on day 11. The Schisandra group (11.07 ± 0.900 g/dl) or combination group (12.13 ± 1.244 g/dl) did not differ significantly from each other or any other group in terms of haemoglobin concentration on day 11. The combination group had higher Hgb values than the control group: however, it was not significant.

It seems possible that CLA stimulated splenic contractions, resulting in a release of erythrocytes into the blood stream, which in turn increased blood haemoglobin concentrations. Increased haemoglobin concentrations in the blood would result in increased performance, as more oxygen would reach the muscles, increasing the muscle capacity to do aerobic work. This was also reported by Lupandin *et al*. (1986). Anaerobic respiration would also decrease if more oxygen reached the muscles, resulting in less lactic acid production.

Leukocyte count

The only significant (P <0.05) difference found in terms of leukocyte count on day 11 was between the CLA treatment group (7.06 ± 1.88 x 10^9/l) and the Schisandra treatment group (4.87 ± 0.50 x 10^9/l). Leukocyte count of the control group (5.60 ± 0.58 x 10^9/l) did not differ significantly from the combination group (5.52 ± 0.98 x 10^9/l), or any other group on day 11.

Leukocyte count of the Schisandra group was slightly lower than the reference range reported by Hadaybit Laboratory, Latimer *et al*. (2003) and Reece (2004).

Multiple factors influence leukocyte production and concentrations; thus, these results should be interpreted with caution.

Differential cell count

No significant differences were found between the four treatment groups in terms of neutrophil, lymphocyte, monocyte, eosinophil or basophil percentages on day 11.

Haematological values of the four groups of horses included in this study are summarized in Table 4.4.11.

Table 4.4.11 Least square means (LSMeans) and standard deviations (SD) of haematological values for endurance horses that received dietary supplements compared to a negative control on day 11 (reference range for adult horses also indicated with reference in italics)

	Blood pH ±SD	Ery ±SD	Hgb ±SD	Leu ±SD	Neu ±SD	Lym ±SD	Mon ±SD	Eos ±SD	Bas ±SD
CLA (n=7)	7.57^{a} ±0.034	7.30^{a} ±0.343	12.43^{a} ±0.522	7.06^{a} ±1.884	59.41 ±4.488	36.73 ±5.577	2.51 ±2.118	1.34 ±0.553	0.00 ±0.000
Control (n=6)	7.49^{b} ±0.021	6.07^{b} ±0.397	10.73^{b} ±0.582	5.60^{ab} ±0.577	56.68 ±9.313	39.07 ±9.958	3.00 ±0.580	1.25 ±0.896	0.00 ±0.000
Schisandra (n=6)	7.51^{b} ±0.032	6.31^{b} ±0.510	11.07^{ab} ±0.900	4.87^{b} ±0.495	54.45 ±3.973	41.37 ±4.816	2.90 ±1.037	1.28 ±0.950	0.00 ±0.000
Combination (n=6)	7.54^{ab} ±0.039	6.84^{ab} ±0.693	12.13^{ab} ±1.244	5.52^{ab} ±0.979	54.33 ±3.586	41.00 ±3.465	3.13 ±1.166	1.25 ±0.616	0.28 ±0.694
Reference range	7.5 – 8.5 *(Hadaybit Laboratory (UAE))*	4.8 – 13 *(Hadaybit Laboratory (UAE))*	9 – 15 *(Hadaybit Laboratory (UAE))*	5 – 11 *(Hadaybit Laboratory (UAE))*	38 – 70 *(Hadaybit Laboratory (UAE))*	25 – 62 *(Hadaybit Laboratory (UAE))*	1 – 8 *(Hadaybit Laboratory (UAE))*	0.1 – 9.5 *(Hadaybit Laboratory (UAE))*	0 – 1.2 *(Hadaybit Laboratory (UAE))*
Reference range		6.0 – 10.4 *(Latimer et al., 2003)*	10 – 16 *(Latimer et al., 2003)*	5.6 - 12.1 *(Latimer et al., 2003)*	52 – 70 *(Latimer et al., 2003)*	21 – 42 *(Latimer et al., 2003)*	0 – 6 *(Latimer et al., 2003)*	0 – 7 *(Latimer et al., 2003)*	0 – 2 *(Latimer et al., 2003)*
Reference range	7.35 – 7.45 *(Davies, 2009)*	7 - 11 *(Reece, 2004)*	11.5 - 16 *(Reece, 2004)*	8 - 11 *(Reece, 2004)*	50 - 60 *(Reece, 2004)*	30 - 40 *(Reece, 2004)*	5 - 6 *(Reece, 2004)*	2 - 5 *(Reece, 2004)*	<1 *(Reece, 2004)*

Ery: Erythrocyte (x 10^{12}g/l); **Hgb:** Haemoglobin (g/dl); **Leu:** Leukocyte (x 10^{9}/l); **Neu:** Neutrophil (%); **Lym:** Lymphocyte (%); **Mon:** Monocyte (%); **Eos:** Eosinophil (%); **Bas:** Basophil (%). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.12 Blood metabolites on Day 11

Creatinine and lactate

No significant differences were found between the four treatment groups in terms of creatinine or lactate concentrations on day 11.

The lactate concentrations of the CLA and combination groups were slightly below that of the reference range reported by Hadaybit Laboratory (UAE), Orsini *et al*. (2008) and Reece (2004). This is probably because the horses were fit and had elevated LDH concentration, which led to the horses being able to break lactate down more effectively.

Lactate dehydrogenase (LDH)

The only observed significant (P <0.05) difference in terms of LDH concentration on day 11 was between the control group (457.33 ± 117.333 U/l) and the combination group (648.67 ± 123.349 U/l). LDH concentration of the CLA (590.14 ± 116.751 U/l) and Schisandra (505.33 ± 81.669 U/l) treatment groups did not differ significantly from each other, or any other group, on day 11.

Both the CLA and Schisandra treatment groups had higher LDH values on day 11 compared to the control group, although neither were significant. The combination group had significantly (P <0.05) higher LDH values, which suggests that the combination of Schisandra and CLA stimulates LDH production. LDH is an enzyme responsible for clearing lactic acid from the body. Horses with higher LDH concentrations in their blood would be able to clear lactic acid from their system more effectively, which could improve performance, as well as aid in the recovery of the body after strenuous exercise.

The LDH concentration of the CLA, Schisandra and combination treatment groups were higher than that of the reference range reported by Hadaybit Laboratory (UAE), Latimer and Prasse (2003) and Orsini *et al*. (2008). As these were fit, hard-working horses at the end of a racing season, they were able to effectively break down lactate in order to recover from strenuous exercise. It can thus be expected that the LDH values will be above the reference range for average horses (not adapted to this intensity of exercise).

Blood urea nitrogen (BUN), cholesterol and LDL

No significant differences were found between the four treatment groups in terms of BUN, cholesterol or LDL concentrations on day 11.

High density lipoprotein (HDL)

The combination group (83.33 ± 5.610 mg/dl) displayed significantly (P <0.05) higher HDL concentrations than any other group on day 11. The CLA (70.43 ± 6.106 mg/dl), control (67.83 ± 12.007 mg/dl) and

Schisandra (68.00 ± 6.753 mg/dl) groups did not differ significantly from each other in terms of HDL concentrations on day 11.This is possibly due to the increased amounts of energy substrates made available to the body by the combination of CLA and Schisandra supplements.

Blood metabolite concentrations of the four groups of horses included in this study are summarized in Table 4.4.12.

Table 4.4.12 Least square means (LSMeans) and standard deviations (SD) of blood metabolites for endurance horses that received dietary supplements compared to a negative control on day 11 (reference range for adult horses also indicated with reference in italics)

	Creatinine ± SD	Lactate ± SD	LDH ± SD	BUN ± SD	Cholesterol ±SD	HDL ±SD	LDL ±SD
CLA (n=7)	1.09 ±0.066	0.81 ±0.224	590.14^{ab} ±116.751	18.90 ±1.758	98.57 ±7.829	70.43^{a} ±6.106	30.71 ±6.601
Control (n=6)	1.05 ±0.133	0.90 ±0.152	457.33^{a} ±117.333	19.12 ±0.875	92.17 ±13.348	67.83^{a} ±12.007	25.33 ±5.203
Schisandra (n=6)	1.10 ±0.222	0.90 ±0.172	505.33^{ab} ±81.669	18.22 ±1.572	96.17 ±6.369	68.00^{a} ±6.753	25.50 ±7.092
Combination (n=6)	1.06 ±0.073	0.82 ±0.155	648.67^{b} ±123.349	17.27 ±1.384	107.00 ±11.189	83.33^{b} ±5.610	24.17 ±12.222
Reference range	0.9 – 2.0 (*Hadaybit Laboratory (UAE)*)	0.9 – 1.7 (*Hadaybit Laboratory (UAE)*)	160 – 500 (*Hadaybit Laboratory (UAE)*)	10 – 24 (*Hadaybit Laboratory (UAE)*)	71 – 142 (*Hadaybit Laboratory (UAE)*)	30 – 90 (*Hadaybit Laboratory (UAE)*)	20 – 40 (*Hadaybit Laboratory (UAE)*)
Reference range	0.4 – 2.2 (*Latimer and Prasse 2003*)		112 – 456 (*Latimer and Prasse 2003*)	11 – 27 (*Latimer and Prasse 2003*)	71 – 142 (*Latimer and Prasse 2003*)		
Reference range	0.9 – 1.9 (*Orsini et al., 2008*)	1.11 – 1.78 (*Orsini et al., 2008*)	162 – 412 (*Orsini et al., 2008*)	12 – 24 (*Orsini et al., 2008*)	75 – 150 (*Orsini et al., 2008*)		
Reference range	1 – 2 (*Reece, 2004*)	1.11 – 1.78 (*Reece, 2004*)		10 – 24 (*Reece, 2004*)	70 – 170 (*CVR Laboratory*)		

Creatinine: mg/dl; **Lactate:** mmol/l; **LDH:** Lactate dehydrogenase (U/l); **BUN:** Blood urea nitrogen (mg/dl); **Cholesterol:** mg/dl; **HDL:** High density lipoprotein (mg/dl); **LDL**: Low density lipoprotein (mg/dl)**.** LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.13 Blood glucose and specific hormone concentrations on Day 11

Glucose

Glucose concentration of the CLA (108.71 ± 6.873 mg/dl) and combination (112.33 ± 11.361 mg/dl) groups did not differ significantly from each other, but both differed significantly (P <0.05) from the control group (136.33 ± 17.328 mg/dl) and Schisandra treatment group (138.33 ± 14.855 mg/dl) on day 11. However, the control and Schisandra groups did not differ significantly from each other in terms of glucose concentration on day 11.

The lower glucose concentration of the CLA and combination groups, compared to the control and Schisandra group, could indicate a better uptake (and use) of glucose from the blood due to an increased rate of metabolism (brought on by the CLA supplement). CLA could also reduce insulin insensitivity, which would result in an increased uptake of glucose from the blood. This improved uptake and utilization of glucose could result in increased performance, as more energy is available to the muscles.

The glucose concentrations of the control and Schisandra groups were higher than the reference range reported by Orsini *et al*. (2008), Hadaybit Laboratory (UAE) and Latimer and Prasse (2003).

Cortisol, T3, T4 and Insulin

No significant differences were found between the four treatment groups in terms of cortisol, T3, T4 or insulin concentrations on day 11.

Blood glucose and specific hormone concentrations of the four groups of horses included in this study are summarized in Table 4.4.13.

Table 4.4.13 Least square means (LSMeans) and standard deviations (SD) of blood glucose and specific hormone concentrations for endurance horses that received dietary supplements compared to a negative control on day 11 (reference range for adult horses also indicated with reference in italics)

	Glucose ±SD	**Cortisol ±SD**	**T3 ±SD**	**T4 ±SD**	**Insulin ±SD**
CLA (n=7)	108.71[a] ±6.873	95.13 ±18.220	0.87 ±0.232	35.48 ±6.352	7.07 ±3.681
Control (n=6)	136.33[b] ±17.328	86.07 ±21.063	0.76 ±0.342	41.26 ±7.623	11.39 ±6.595
Schisandra (n=6)	138.33[b] ±14.855	71.45 ±22.644	0.69 ±0.224	40.52 ±6.914	10.36 ±8.276
Combination (n=6)	112.33[a] ±11.361	79.62 ±29.893	0.62 ±0.114	30.59 ±7.211	5.72 ±5.906
Reference range	75 – 115 *(Orsini et al., 2008)*				10 – 30 *(Orsini et al., 2008)*
Reference range		69 – 180 *(CVR Laboratory)*	0.24 – 1.28 *(CVR Laboratory)*	16.5 – 24.4 *(CVR Laboratory)*	5.7 – 31.4 *(CVR Laboratory)*
Reference range	62 – 114 *(Hadaybit Laboratory (UAE))*	90 – 200 *(Idexx Laboratory (RSA))*	0.5 – 2.5 *(Idexx Laboratory (RSA))*	11 – 37 *(Idexx Laboratory (RSA))*	4 – 20 *(Idexx Laboratory (RSA))*
Reference range	62 – 134 *(Latimer and Prasse, 2003)*		0.3 – 2.9 *(Robinson & Sprayberry, 2009)*	6 – 46 *(Robinson & Sprayberry, 2009)*	

Glucose: mg/dl; **Cortisol:** nmol/l; **T3:** Triiodothyronine (nmol/l); **T4:** Thyroxin (nmol/l); **Insulin:** (µlU/l). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

Day 15: Supplementation

Mid-experimental phase: in order to evaluate the medium-term effects that the supplements had on the heart and respiration rates (both pre- and post exercise) of horses.

4.4.14 Pre-exercise basal physiological parameters on Day 15

Basal heart rate

The only observed significant (P <0.05) difference in terms of basal heart rate (beats per minute/bpm) on day 15 was between the CLA treatment group (33.9 ± 3.89 bpm) and the Schisandra treatment group (25.8 ± 3.37 bpm). These two groups did not differ significantly from the control (30.7 ± 6.83 bpm) or combination (28.2 ± 2.32 bpm) groups. Basal heart rate of the control and combination groups also did not differ significantly from each other.

The CLA treatment group displayed increased basal heart rate values, but this was significant only when compared to the Schisandra treatment group. This could be due to a higher metabolism brought on by CLA supplementation. Schisandra had an effect on lowering the basal heart rate (both when supplemented alone or in combination with CLA) when compared to the control group, but this was not significant. The effects of the two different supplements (CLA and Schisandra) are opposite, with CLA increasing basal heart rate, and Schisandra decreasing basal heart rate (neither was significant when compared to the control group).

Basal respiration rate

No significant differences were found between the four treatment groups in terms of basal respiration rates (inhalations per minute/ipm) on day 15.

Basal temperature (rectal)

No significant (P <0.05) differences were found between the four treatment groups in terms of basal rectal temperatures on day 15. All four groups' rectal temperatures were slightly below the reference range reported by Huntington *et al*. (2004) and Robertshaw (2004). This could be because of cool winter climates and early morning temperature recordings.

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 4.4.14.

Table 4.4.14 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control on day 15 (reference range for adult horses also indicated with reference in italics)

	Basal HR ± SD	**Basal Resp ± SD**	**Basal Temp ± SD**
CLA (n=7)	33.9[a] ±3.89	11.4 ±1.90	36.3 ±0.37
Control (n=6)	30.7[ab] ±6.83	11.7 ±2.94	36.2 ±0.36
Schisandra (n=6)	25.8[b] ±3.37	12.3 ±2.94	36.0 ±0.80
Combination (n=6)	28.2[ab] ±2.32	12.7 ±1.63	36.4 ±0.43
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington et al., 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.15 Post exercise parameters on Day 15

Heart rates post exercise

No significant differences were found between the four treatment groups in terms of heart rate at 0, 10 or 20 minutes post exercise on day 15.

Trendlines for these results are presented in Figure 4.4.7.

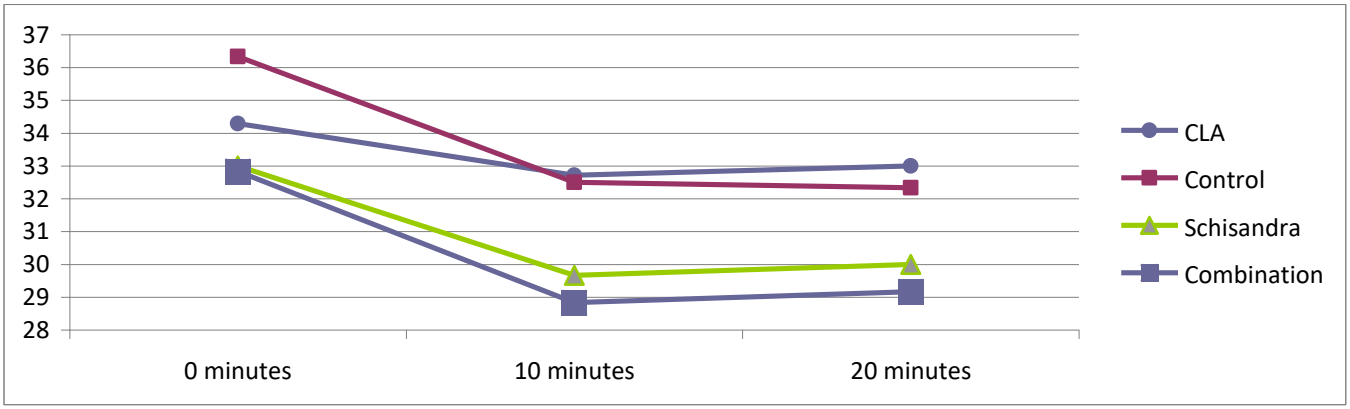

Figure 4.4.7 Changes in heart rate (beats per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 15

Respiration rates post exercise

No significant differences were found between the four treatment groups in terms of respiration rate at 0, 10 or 20 minutes post exercise on day 15.

Trendlines for these results are presented in Figure 4.4.8.

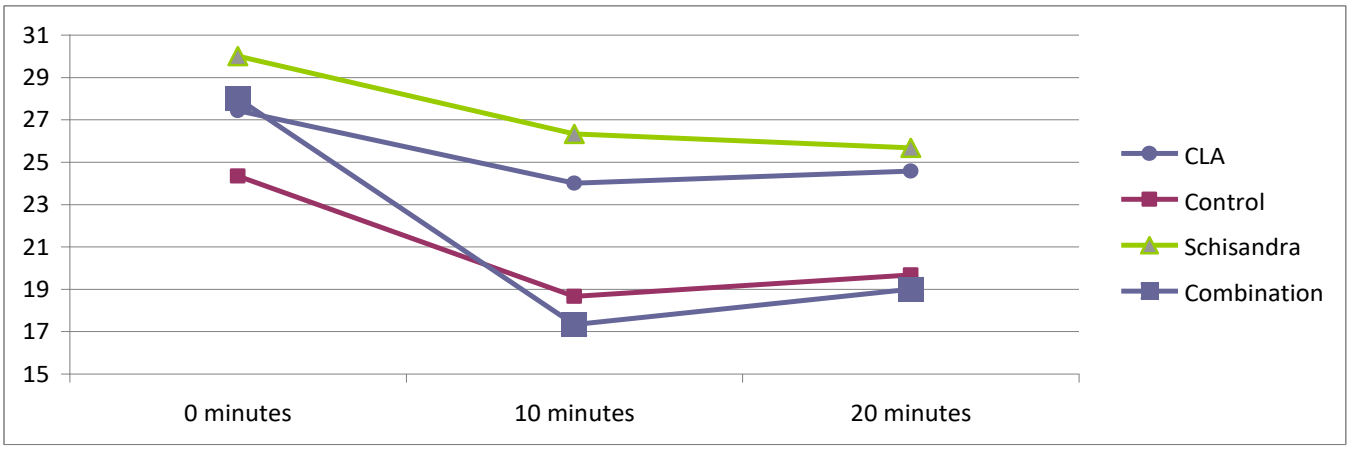

Figure 4.4.8 Changes in respiration rate (inhalations per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 15

Post exercise parameters of the four groups of horses included in this study are summarized in Table 4.4.15.

Table 4.4.15 Least square means (LSMeans) and standard deviations (SD) of post exercise parameters for endurance horses that received dietary supplements compared to a negative control on day 15

	HR 0 min ± SD	HR 10 min ± SD	HR 20 min ± SD	Resp 0 min ± SD	Resp 10 min ± SD	Resp 20 min ± SD
CLA (n=7)	34.3 ±4.19	32.7 ±2.81	33.0 ±4.32	27.4 ±6.50	24.0 ±6.93	24.6 ±5.00
Control (n=6)	36.3 ±5.01	32.5 ±4.93	32.3 ±4.27	24.3 ±9.16	18.7 ±6.65	19.7 ±6.86
Schisandra (n=6)	33.0 ±4.34	29.7 ±2.42	30.0 ±5.55	30.0 ±5.66	26.3 ±6.50	25.7 ±7.94
Combination (n=6)	32.8 ±3.54	28.8 ±2.93	29.2 ±2.48	28.0 ±3.35	17.3 ±2.42	19.0 ±2.76

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm) 20 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. LSMeans with different superscript letters in the same columns differed (P <0.05).

Day 19: End of supplementation

The goal was to evaluate the long term supplementation effect of these supplements and to determine whether the observed changes increased during the duration of the supplementation period, or whether the effects of the different supplements diminished over time.

4.4.16 Pre-exercise basal physiological parameters on Day 19

Basal heart rate and rectal temperature

No significant differences were found between the four treatment groups in terms of basal heart rate (beats per minute/bpm) or basal rectal temperature on day 19.

Basal respiration rate

Basal respiration rate (inhalations per minute/ipm) of the combination group (24.0 ± 3.46 ipm) differed significantly (P <0.05) from all three other groups on day 19. The CLA (18.0 ± 2.31 ipm), control (16.3 ± 1.97 ipm) and Schisandra (15.3 ± 3.93 ipm) groups did not differ significantly from each other in terms of basal respiration rate on day 19.

The combination group showed increased basal respiration above that of when its components (CLA and Schisandra) were fed separately to the horses.

Basal heart rates, respiration rates and rectal temperature of the four groups of horses included in this study are summarized in Table 4.4.16.

Table 4.4.16 Least square means (LSMeans) and standard deviations (SD) of basal heart rate, respiration rate and rectal temperature for endurance horses that received dietary supplements compared to a negative control on day 19 (reference range for adult horses also indicated with reference in italics)

	Basal HR ± SD	**Basal Resp ± SD**	**Basal Temp ± SD**
CLA (n=7)	30.4 ±5.19	18.0[a] ±2.31	37.6 ±0.10
Control (n=6)	31.2 ±3.60	16.3[a] ±1.97	37.1 ±0.54
Schisandra (n=6)	28.7 ±5.16	15.3[a] ±3.93	37.3 ±0.28
Combination (n=5)	29.8 ±2.28	24.0[b] ±3.46	37.4 ±0.24
Reference range	30 – 40 *(Huntington et al., 2004)*	8 – 20 *(Huntington* et al.*, 2004)*	37.0 – 38.0 *(Huntington et al., 2004)*
Reference range	28 - 40 *(Detweiler & Erickson, 2004)*	10 – 14 *(Reece, 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*
Reference range	25 – 40 *(Evans, 1994)*		

Basal HR: Basal heart rate (beats per minute (bpm)); **Basal Resp:** Basal respiration rate (inhalations per minute (ipm)); **Basal Temp:** Basal temperature (rectal) in degrees Celsius (°C). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.17 Post exercise parameters on Day 19

Heart rates 0 and 10 minutes post exercise

No significant differences were found between the four treatment groups in terms of heart rate at 0 or 10 minutes post exercise on day 19.

Heart rate 20 minutes post exercise

The only observed significant (P <0.05) difference in terms of heart rate, 20 minutes post exercise on day 19, was between the control group (35.8 ± 4.45 bpm) and the Schisandra treatment group (28.3 ± 2.07 bpm). These two groups did not differ significantly from the CLA (32.1 ± 1.57 bpm) or combination (31.8 ± 2.17

bpm) treatment groups. The CLA and combination treatment groups did not differ significantly from each other with regard to heart rate, 20 minutes post exercise on day 19.

Schisandra, when supplemented on its own, drastically reduced the heart rate values at 20 minutes post exercise. The other two treatment groups (CLA and combination) displayed lower heart rate values compared to the control group, but this was not significant.

Trendlines for these results are presented in Figure 4.4.9.

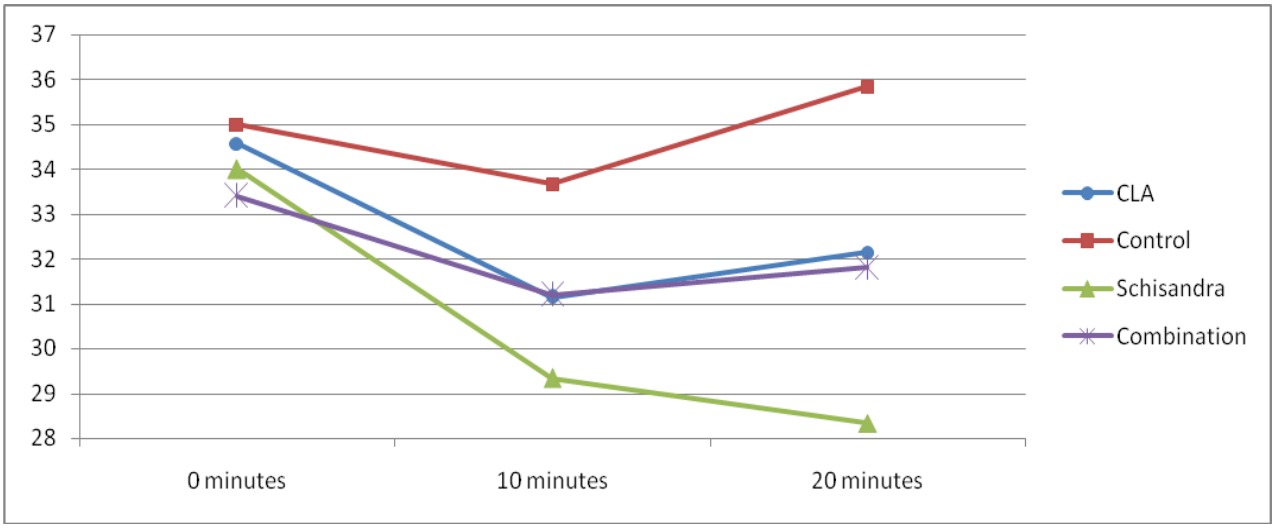

Figure 4.4.9 Changes in heart rate (beats per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 19

Respiration rates post exercise

No significant differences were found between the four treatment groups in terms of respiration rate at 0, 10 or 20 minutes post exercise on day 19.

Trendlines for these results are presented in Figure 4.4.10.

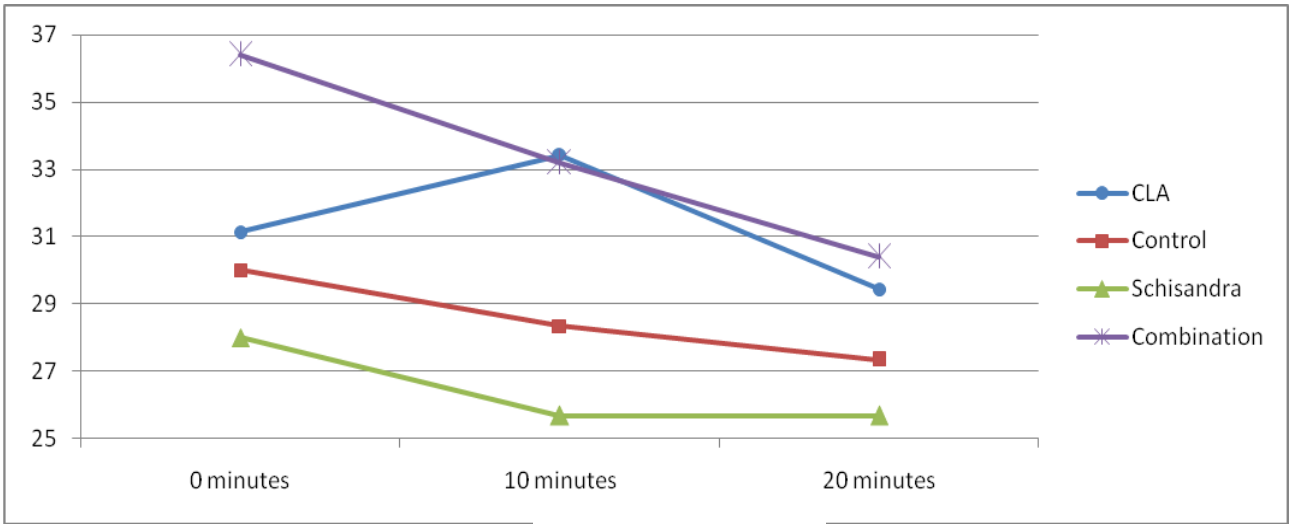

Figure 4.4.10 Changes in respiration rate (inhalations per minute) of the four treatment groups at 0, 10 and 20 minutes post exercise on Day 19

Post exercise parameters of the four groups of horses included in this study are summarized in Table 4.4.17.

Table 4.4.17 Least square means (LSMeans) and standard deviations (SD) of post exercise parameters for endurance horses that received dietary supplements compared to a negative control on day 19

	HR 0 min ± SD	HR 10 min ± SD	HR 20 min ± SD	Resp 0 min ± SD	Resp 10 min ± SD	Resp 20 min ± SD
CLA (n=7)	34.6 ±2.76	31.1 ±2.73	32.1^{ab} ±1.57	31.1 ±3.02	33.4 ±7.00	29.4 ±4.58
Control (n=6)	35.0 ±3.16	33.7 ±3.14	35.8^{a} ±4.45	30.0 ±8.58	28.3 ±9.07	27.3 ±3.93
Schisandra (n=6)	34.0 ±4.56	29.3 ±2.07	28.3^{b} ±2.07	28.0 ±8.94	25.7 ±5.99	25.7 ±9.42
Combination (n=5)	33.4 ±1.52	31.2 ±3.27	31.8^{ab} ±2.17	36.4 ±2.19	33.2 ±3.90	30.4 ±5.18

Exercise HR 0min: Heart rate (beats per minute (bpm)) recorded 0 minutes after exercise; **Exercise HR 10min:** Heart rate (bpm) recorded 10 minutes after exercise; **Exercise HR 20min:** Heart rate recorded (bpm) 20 minutes after exercise ; **Exercise Resp 0min:** Respiration rate (inhalations per minute (ipm)) recorded 0 minutes after exercise; **Exercise Resp 10min:** Respiration rate (ipm) recorded 10 minutes after exercise; **Exercise Resp 20min:** Respiration rate (ipm) recorded 20 minutes after exercise. LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.18 Haematological values on Day 19

Blood pH and erythrocyte count

No significant differences were found between the four treatment groups in terms of blood pH or erythrocyte count on day 19.

On day 19, all groups' erythrocyte count was slightly lower than the reference range reported by Hadaybit Laboratory (UAE), Latimer *et al*. (2003) and Reece (2004).

Haemoglobin (Hgb)

No significant differences were found between the four treatment groups in terms of haemoglobin concentration on day 19. The haemoglobin concentration of the combination group was slightly higher than that of the reference range reported by Hadaybit Laboratory (UAE), Latimer *et al*. (2003) and Reece (2004); however, it was not significantly higher than any of the other three treatment groups.

Leukocyte count

No significant differences were found between the four treatment groups in terms of leukocyte count on day 19.

Differential cell count

No significant differences were found between the four treatment groups in terms of neutrophil, lymphocyte, monocyte, eosinophil or basophil percentages on day 19.

Haematological values of the four groups of horses included in this study are summarized in Table 4.4.18.

Table 4.4.18 Least square means (LSMeans) and standard deviations (SD) of haematological values for endurance horses that received dietary supplements compared to a negative control on day 19 (reference range for adult horses also indicated with reference in italics)

	Blood pH ±SD	**Leu ±SD**	**Ery ±SD**	**Hgb ±SD**	**Neu ±SD**	**Lym ±SD**	**Mon ±SD**	**Eos ±SD**	**Bas ±SD**
CLA (n=7)	7.53 ±0.029	6.78 ±1.739	6.91 ±0.652	11.77 ±0.959	58.43 ±4.317	37.23 ±5.182	3.07 ±1.591	1.27 ±0.509	0.00 ±0.000
Control (n=6)	7.51 ±0.044	6.88 ±0.715	6.34 ±0.332	11.32 ±0.407	55.88 ±9.188	40.18 ±9.417	2.90 ±0.616	0.95 ±0.464	0.08 ±0.204
Schisandra (n=6)	7.53 ±0.034	5.83 ±0.748	6.50 ±0.467	11.50 ±1.055	54.10 ±5.763	42.33 ±6.669	2.28 ±1.264	1.28 ±0.970	0.00 ±0.000
Combination (n=5)	7.52 ±0.020	6.67 ±1.227	6.96 ±0.590	12.50 ±0.982	57.28 ±3.924	38.76 ±4.180	2.16 ±0.844	1.42 ±1.083	0.38 ±0.850
Reference range	7.5 – 8.5 *(Hadaybit Laboratory (UAE))*	4.8 – 13 *(Hadaybit Laboratory (UAE))*	9 – 15 *(Hadaybit Laboratory (UAE))*	5 – 11 *(Hadaybit Laboratory (UAE))*	38 – 70 *(Hadaybit Laboratory (UAE))*	25 – 62 *(Hadaybit Laboratory (UAE))*	1 – 8 *(Hadaybit Laboratory (UAE))*	0.1 – 9.5 *(Hadaybit Laboratory (UAE))*	0 – 1.2 *(Hadaybit Laboratory (UAE))*
Reference range		6.0 – 10.4 *(Latimer et al., 2003)*	10 – 16 *(Latimer et al., 2003)*	5.6 - 12.1 *(Latimer et al., 2003)*	52 – 70 *(Latimer et al., 2003)*	21 – 42 *(Latimer et al., 2003)*	0 – 6 *(Latimer et al., 2003)*	0 – 7 *(Latimer et al., 2003)*	0 – 2 *(Latimer et al., 2003)*
Reference range	7.35 – 7.45 *(Davies, 2009)*	7 - 11 *(Reece, 2004)*	11.5 - 16 *(Reece, 2004)*	8 - 11 *(Reece, 2004)*	50 - 60 *(Reece, 2004)*	30 - 40 *(Reece, 2004)*	5 - 6 *(Reece, 2004)*	2 - 5 *(Reece, 2004)*	<1 *(Reece, 2004)*

Ery: Erythrocyte (x 10^{12}g/l); **Hgb:** Haemoglobin (g/dl); **Leu:** Leukocyte (x 10^{9}/l); **Neu:** Neutrophil (%); **Lym:** Lymphocyte (%); **Mon:** Monocyte (%); **Eos:** Eosinophil (%); **Bas:** Basophil (%). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.19 Blood metabolites on Day 19

Creatinine and lactate

No significant differences were found between the four treatment groups in terms of creatinine or lactate concentration on day 19.

The lactate concentrations of the CLA and Schisandra groups were slightly below that of the reference range reported by Hadaybit Laboratory (UAE), Orsini *et al*. (2008) and Reece (2004). This is probably because the horses were fit and had elevated LDH concentration, which led to the horses being able to metabolise lactate more effectively.

Lactate Dehydrogenase (LDH)

The control group (484.00 ± 107.685 U/l) differed significantly (P <0.05) from the combination treatment group (714.80 ± 166.348 U/l) in terms of LDH concentration on day 19. These two groups did not differ significantly from the CLA (603.00 ± 110.817 U/l) or Schisandra (551.50 ± 76.524 U/l) treatment groups, and the CLA and Schisandra treatment groups did not differ significantly from each other with regard to LDH concentration on day 19.

The CLA and Schisandra groups displayed higher LDH values compared to the control group, but not significantly so. The combination group displayed the highest LDH concentration, significantly higher than that of the control group. This suggests that the combination of Schisandra and CLA stimulates LDH production. LDH is an enzyme responsible for clearing lactic acid from the body. Horses with higher LDH concentration in their blood would be able to clear lactic acid from their system more effectively, which could improve performance, as well as aid in the recovery of the body after strenuous exercise.

The LDH concentration of the CLA, Schisandra and combination treatment groups were higher than that of the reference range reported by Hadaybit Laboratory (UAE), Latimer and Prasse (2003) and Orsini *et al*. (2008). As these were fit, hard-working horses at the end of a racing season, they were able to effectively break down lactate in order to recover from strenuous exercise. It can thus be expected that the LDH values will be above the reference range for average horses (not adapted to this intensity of exercise).

Blood urea nitrogen (BUN), total cholesterol and LDL

No significant differences were found between the four treatment groups in terms of BUN, cholesterol and LDL concentrations on day 19.

High density lipoprotein (HDL)

HDL concentrations of the combination treatment group (84.80 ± 9.497 mg/dl) differed significantly (P <0.05) from both the control (68.33 ± 11.656 mg/d) and Schisandra (67.83 ± 7.627 mg/dl) treatment groups on day 19, but not from the CLA treatment group (70.43 ± 6.828 mg/dl). The control and Schisandra treatment group did not differ significantly from each other or from the CLA group with regard to HDL concentration on day 19.

It is clear that CLA increases the HDL cholesterol concentration significantly (P <0.05), also when fed together with Schisandra (as in the combination group).

It is interesting to note that no significant differences were found in terms of total cholesterol values on Day 11 or 19. The variation observed between the four groups regarding LDL concentration had also stabilized, as the LDL values are more similar on Day 19 compared to Day 0 and Day 11.

Blood metabolite concentrations of the four groups of horses included in this study are summarized in Table 4.4.19.

Table 4.4.19 Least square means (LSMeans) and standard deviations (SD) of blood metabolites for endurance horses that received dietary supplements compared to a negative control on day 19 (reference range for adult horses also indicated with reference in italics)

	Creatinine ± SD	**Lactate ± SD**	**LDH ± SD**	**BUN ± SD**	**Cholesterol ±SD**	**HDL ±SD**	**LDL ±SD**
CLA (n=7)	1.23 ±0.065	0.76 ±0.379	603.00^{ab} ±110.817	25.21 ±1.224	95.71 ±6.626	70.43^{ab} ±6.828	28.71 ±6.157
Control (n=6)	1.18 ±0.097	0.93 ±0.270	484.00^{a} ±107.685	23.92 ±1.752	90.17 ±12.734	68.33^{a} ±11.656	25.50 ±4.550
Schisandra (n=6)	1.24 ±0.186	0.88 ±0.30	551.50^{ab} ±76.524	23.85 ±1.523	93.50 ±8.167	67.83^{a} ±7.627	27.67 ±4.885
Combination (n=5)	1.23 ±0.104	1.06 ±0.295	714.80^{b} ±166.348	24.08 ±0.572	107.20 ±12.029	84.80^{b} ±9.497	29.60 ±11.610
Reference range	0.9 – 2.0 *(Hadaybit Laboratory (UAE))*	0.9 – 1.7 *(Hadaybit Laboratory (UAE))*	160 – 500 *(Hadaybit Laboratory (UAE))*	10 – 24 *(Hadaybit Laboratory (UAE))*	71 – 142 *(Hadaybit Laboratory (UAE))*	30 – 90 *(Hadaybit Laboratory (UAE))*	20 – 40 *(Hadaybit Laboratory (UAE))*
Reference range	0.4 – 2.2 *(Latimer and Prasse, 2003)*		112 – 456 *(Latimer and Prasse, 2003)*	11 – 27 *(Latimer and Prasse, 2003)*	71 – 142 *(Latimer and Prasse, 2003)*		
Reference range	0.9 – 1.9 *(Orsini et al., 2008)*	1.11 – 1.78 *(Orsini et al., 2008)*	162 – 412 *(Orsini et al., 2008)*	12 – 24 *(Orsini et al., 2008)*	75 – 150 *(Orsini et al., 2008)*		
Reference range	1 – 2 *(Reece, 2004)*	1.11 – 1.78 *(Reece, 2004)*		10 – 24 *(Reece, 2004)*	70 – 170 *(CVR Laboratory)*		

Creatinine: mg/dl; **Lactate:** mmol/l; **LDH:** Lactate dehydrogenase (U/l); **BUN:** Blood urea nitrogen (mg/dl); **Cholesterol:** mg/dl; **HDL:** High density lipoprotein (mg/dl); **LDL**: Low density lipoprotein (mg/dl)**.** (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.4.20 Blood glucose and specific hormone concentrations on Day 19

No significant differences were found between the four treatment groups in terms of blood glucose, cortisol, T3, T4 or insulin concentration on day 19.

The insulin concentrations of the CLA control groups were slightly below the reference range reported by Orsini et al. (2008), CVR Laboratory and Idexx Laboratory (RSA). As insulin is quite unstable, it is possible that some of the insulin was broken down prior to analysis, and these results should be interpreted with caution.

Blood glucose and specific hormone concentrations of the four groups of horses included in this study are summarized in Table 4.4.20.

Table 4.4.20. Least square means (LSMeans) and standard deviations (SD) of blood glucose and specific hormone concentrations for endurance horses that received dietary supplements compared to a negative control on day 19 (reference range for adult horses also indicated with reference in italics)

	Glucose ±SD	**Cortisol ±SD**	**T3 ±SD**	**T4 ±SD**	**Insulin ±SD**
CLA (n=7)	101.14 ±4.140	100.79 ±31.605	0.61 ±0.130	29.07 ±6.468	3.65 ±1.864
Control (n=6)	102.17 ±14.049	110.52 ±22.074	0.57 ±0.047	34.28 ±10.214	2.99 ±1.507
Schisandra (n=6)	103.33 ±9.416	122.95 ±28.910	0.60 ±0.089	35.02 ±7.228	4.74 ±4.322
Combination (n=5)	107.00 ±13.638	109.26 ±27.470	0.64 ±0.103	26.33 ±5.810	4.12 ±2.513
Reference range	75 – 115 *(Orsini et al., 2008)*				10 – 30 *(Orsini et al., 2008)*
Reference range		69 – 180 *(CVR Laboratory)*	0.24 – 1.28 *(CVR Laboratory)*	16.5 – 24.4 *(CVR Laboratory)*	5.7 – 31.4 *(CVR Laboratory)*
Reference range	62 – 114 *(Hadaybit Laboratory (UAE))*	90 – 200 *(Idexx Laboratory (RSA))*	0.5 – 2.5 *(Idexx Laboratory (RSA))*	11 – 37 *(Idexx Laboratory (RSA))*	4 – 20 *(Idexx Laboratory (RSA))*
Reference range	62 – 134 *(Latimer and Prasse, 2003)*		0.3 – 2.9 *(Robinson & Sprayberry, 2009)*	6 – 46 *(Robinson & Sprayberry, 2009)*	

Glucose: mg/dl; **Cortisol:** nmol/l; **T3:** Triiodothyronine (nmol/l); **T4:** Thyroxin (nmol/l); **Insulin:** (µlU/l). (*Reference indicated in italics).* LSMeans with different superscript letters in the same columns differed (P <0.05).

4.5 Comparisons between initial (Day 0) and end (Day 19) physiological parameters in each group

Mean end values (day 19) were compared to the mean baseline values (day 0) for each group in order to evaluate the effect of long term supplementation within each specific group.

4.5.1 Basal respiration rate, day 0 versus day 19

Both the CLA and combination treatment groups' basal respiration rate increased significantly (P <0.05) from day 0 to day 19, which was also the case for the control group. It is likely that this increase in basal respiration rate was due to an anticipation effect. As the horses became more accustomed to the daily routine they became more excited in anticipation of the exercise routine. It is interesting to note that the Schisandra group's basal respiration rate did not increase significantly (P <0.05) between day 0 and day 19, as opposed to the other treatment groups where a significant (P <0.05) increase in basal respiration rates was noted. Basal respiration rate on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.1.

Table 4.5.1 Least square means (LSMeans) and standard deviations (SD) of basal respiration rate (inhalations per minute (ipm)) for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	13.0 [a] ± 1.63	18.0 [b] ± 2.31
Control	12.8 [a] ± 1.83	16.3 [b] ± 1.97
Schisandra	13.5 ± 2.17	15.3 ± 3.93
Combination	13.2 [a] ± 1.789	24.0 [b] ± 3.46
Overall Mean	13.1 ± 0.66	18.2 ± 0.66
Reference ranges (overall)	8 – 20 *(Huntington et al., 2004)*	10 – 14 *(Reece, 2004)*

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean. (*Reference indicated in italics).*

4.5.2 Basal temperature, day 0 versus day 19

Basal temperature of the CLA, Schisandra and combination groups increased significantly (P <0.05) from day 0 to day 19. This could be the result of increased metabolism brought about by the supplements fed to

the horses (CLA, Schisandra and the combination of these two supplements). Basal temperature of the control group did not increase significantly (P <0.05) between day 0 and day 19.

Basal temperature on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.2.

Table 4.5.2 Least square means (LSMeans) and standard deviations (SD) of basal temperature (degrees Celsius (ºC)) for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	36.51 [a] ± 0.275	37.56 [b] ± 0.102
Control	36.61 ± 0.462	37.09 ± 0.543
Schisandra	36.32 [a] ± 0.642	37.28 [b] ± 0.280
Combination	36.72 [a] ± 0.407	37.38 [b] ± 0.238
Overall Mean	36.53 ± 0.454	37.34 ± 0.356
Reference ranges (overall)	37.0 – 38.0 *(Huntington et al., 2004)*	37.2 – 38.2 *(Robertshaw, 2004)*

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean. (*Reference indicated in italics).*

4.5.3 Heart rate, 20 minutes post exercise, day 0 versus day 19

Only the control group displayed significant (P <0.05) increase in heart rate at 20 minutes post exercise between day 0 and day 19. As the control group did not receive any supplements, it is not clear why this occurred. It is possible that these supplements stimulated faster recovery post exercise, thus no significant (P <0.05) differences in heart rate at 20 minutes post exercise were observed for the treatment groups which were fed supplements (CLA, Schisandra or combination).

Heart rate, 20 minutes post exercise on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.3.

Table 4.5.3 Least square means (LSMeans) and standard deviations (SD) of heart rate 20 minutes post exercise (beats per minute (bpm)) for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	31.0 ± 4.65	32.1 ± 1.57
Control	27.7 [a] ± 4.72	35.8 [b] ± 4.45
Schisandra	29.3 ± 8.80	28.3 ± 2.07
Combination	30.6 ± 3.13	31.8 ± 2.17
Overall Mean	29.7 ± 5.55	32.0 ± 3.75

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean.

4.5.4 Respiration rate, 0 minutes post exercise, day 0 versus day 19

Both CLA and control groups displayed significantly (P <0.05) higher respiration rates 0 minutes post exercise on day 19 compared to day 0. The Schisandra and combination groups' respiration rate at 0 minutes post exercise did not increase significantly (P <0.05) from day 0 to day 19. It is possible that this significant (P <0.05) increase in respiration at 0 minutes post exercise was due to high temperatures on day 19. It is interesting to note that both groups which received Schisandra supplements (Schisandra and combination groups) did not display significantly (P <0.05) higher respiration rates at 0 minutes post exercise on day 19 compared to day 0. It is likely that this increase in respiration rate at 0 minutes post exercise on day 19 was prevented by the adaptogen-like properties of Schisandra.

Respiration rate, 0 minutes post exercise on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.4.

Table 4.5.4 Least square means (LSMeans) and standard deviations (SD) of respiration rate (inhalations per minute (ipm)) at 0 minutes post exercise for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	20.6 [a] ± 4.76	31.1 [b] ± 3.02
Control	15.3 [a] ± 4.37	30.0 [b] ± 8.58
Schisandra	27.7 ± 9.83	28.0 ± 8.94
Combination	34.4 ± 5.37	36.4 ± 2.19
Overall Mean	23.9 ± 1.66	31.2 ± 1.66

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean.

4.5.5 Respiration rate, 10 minutes post exercise, day 0 versus day 19

Similar to respiration rate at 0 minutes post exercise, both CLA and control groups displayed significantly (P <0.05) higher respiration rates 10 minutes post exercise on day 19 compared to day 0. Once again the Schisandra and combination groups' respiration rate at 10 minutes post exercise did not increase significantly (P <0.05) from day 0 to day 19.

Respiration rate, 10 minutes post exercise on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.5.

Table 4.5.5 Least square means (LSMeans) and standard deviations (SD) of respiration rate (inhalations per minute (ipm)) at 10 minutes post exercise for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	20.7 [a] ± 1.11	33.4 [b] ± 6.70
Control	14.2 [a] ± 3.71	28.3 [b] ± 9.07
Schisandra	22.0 ± 6.57	25.7 ± 5.99
Combination	25.6 ± 8.29	33.2 ± 3.90
Overall Mean	20.417 ± 1.398	30.167 ± 1.398

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean.

4.5.6 Respiration rate, 20 minutes post exercise, day 0 versus day 19

Again the only significant (P <0.05) difference with regards to respiration rate at 20 minutes post exercise was seen for the CLA and combination groups. No significant (P <0.05) differences for respiration rate at 20 minutes post exercise were seen for the Schisandra and combination groups. It appears as if Schisandra supplementation (alone or in combination with CLA) prevented increase in respiration rate on day 19 compared to day 0 at 0, 10 and 20 minutes post exercise. This in most likely due to the adaptogen-like effect of Schisandra. Horses which received Schisandra supplement (Schisandra and combination groups) were better adapted on day 19 compared to the other two treatment groups.

Respiration rate, 20 minutes post exercise on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.6.

Table 4.5.6 Least square means (LSMeans) and standard deviations (SD) of respiration rate (inhalations per minute (ipm)) at 20 minutes post exercise for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	22.9 [a] ± 3.76	29.4 [b] ± 4.58
Control	14.0 [a] ± 3.95	27.3 [b] ± 3.93
Schisandra	19.3 ± 7.34	25.7 ± 9.42
Combination	22.4 ± 6.07	30.4 ± 5.18
Overall mean	19.7 ± 1.25	28.2 ± 1.25

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean.

4.5.7 Monocyte concentration, day 0 versus day 19

Only the control group displayed a significant (P <0.05) increase in monocyte concentration from day 0 to day 19. As the control group did not receive any supplements, it is not clear why this occurred. Monocyte concentration on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.7.

Table 4.5.7 Least square means (LSMeans) and standard deviations (SD) of monocyte concentration (%) for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	3.07 ± 2.232	3.07 ± 1.591
Control	1.70 [a] ± 0.700	2.90 [b] ± 0.616
Schisandra	2.07 ± 1.035	2.28 ± 1.264
Combination	2.86 ± 1.258	2.16 ± 0.844
Overall Mean	2.43 ± 1.492	2.64 ± 1.171
Reference ranges (overall)	1 – 8 *(Hadaybit Laboratory (UAE))*	0 – 6 *(Latimer et al., 2003)*

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean. (*Reference indicated in italics).*

4.5.8 Creatinine concentration, day 0 versus day 19

CLA, control and combination groups displayed significantly (P <0.05) higher creatinine concentrations on day 19 compared to day 0. This could be due to an increased protein turnover as a result of increased metabolism and fitness levels. The creatinine concentration of the Schisandra group did not increase significantly (P <0.05), however when looking at the mean values on day 0 versus day 19, a similar numerical distance is seen in this group compared to the other three groups. The reason why these two days did not differ significantly for the Schisandra treatment group is probably due to the large standard deviations on both day 0 and day 19. These large standard deviations result in an overlap of the values of day 0 and day 19, thus no significant difference is found for the Schisandra treatment group. This could be due to an outlier horse.

Creatinine concentration on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.8.

Table 4.5.8 Least square means (LSMeans) and standard deviations (SD) of creatinine concentration (mg/dl) for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	1.05 [a] ± 0.081	1.23 [b] ± 0.065
Control	0.99 [a] ± 0.096	1.18 [b] ± 0.097
Schisandra	1.08 ± 0.179	1.24 ± 0.186
Combination	1.01 [a] ± 0.033	1.23 [b] ± 0.104
Overall Mean	1.03 ± 0.111	1.22 ± 0.115
Reference ranges (overall)	0.4 – 2.2 *(Latimer and Prasse, 2003)*	0.9 – 1.9 *(Orsini et al., 2008)*

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean. (*Reference indicated in italics).*

4.5.9 Blood urea nitrogen (BUN) concentration, day 0 versus day 19

BUN concentration of all four treatment groups decreased significantly (P <0.05) from day 0 to day 19. It is unclear why this occurred as the control group, who only received a placebo, also displayed significantly (P <0.05) lower BUN concentrations on day 19 compared to day 0. It is likely that in all four treatment groups BUN sources were utilised more efficiently for protein synthesis as a result of increased fitness levels.

Blood urea nitrogen (BUN) concentration on day 0 versus day 19 for the four groups of horses included in this study are summarized in Table 4.5.9.

Table 4.5.9 Least square means (LSMeans) and standard deviations (SD) of blood urea nitrogen (BUN) concentration (mg/dl) for endurance horses that received dietary supplements (Comparisons between results of day 0 versus day 19)

Group	Day 0	Day 19
CLA	29.71 [a] ± 0.997	25.21 [b] ± 1.224
Control	29.38 [a] ± 2.197	23.92 [b] ± 1.752
Schisandra	28.73 [a] ± 1.766	23.85 [b] ± 1.523
Combination	26.94 [a] ± 0.568	24.08 [b] ± 0.572
Overall Mean	28.81 ± 0.326	24.31 ± 0.326
Reference ranges (overall)	11 – 27 *(Latimer and Prasse, 2003)*	12 – 24 *(Orsini et al., 2008)*

LSMeans with different superscript letters in the same rows differed (P <0.05). **LSMean:** least square mean. (*Reference indicated in italics).*

No significant differences were found between day 0 and day 19 in each group for the following parameters:

- Basal heart rate
- Heart rate at 0 minutes post exercise
- Heart rate at 10 minutes post exercise
- Blood pH
- White blood cell (WBC) and red blood cell (RBC) count
- Haemoglobin concentration
- Neutrophil, lymphocyte, eosinophil and basophil
- Glucose, cholesterol, HDL and LDL

- Lactate, lactate dehydrogenase, cortisol, T3, T4 and insulin

Plasma and Urine Schizandrin concentration

The Schizandrin and its metabolite deoxyschizandrin concentration in both the urine and plasma samples were below the detectable range (minimum detectable range 50µg/l).

Chapter 5

Summary and conclusion

5.1.1 Summary of the pooled results

Basal heart rate

Schisandra (supplemented on its own or in combination with CLA) significantly (P <0.05) decreased the basal heart rate of horses in the Schisandra and combination treatment groups over the entire trial period, which corresponds with the findings of Hancke *et al*. (1994).

Basal respiration rate

When supplemented on its own, CLA significantly increased basal respiration rate of horses compared to those in the control group. The higher basal respiration rate is probably due to a higher metabolic rate brought on by CLA supplementation. A higher basal respiration rate would result in more oxygen reaching the muscles, and, therefore, less lactic acid production, which increases the ability of muscles to perform work.

Basal temperature (rectal)

Dietary Schisandra supplementation resulted in slightly lower rectal temperature values in the Schisandra treated horses (pooled results), but at this stage it is not clear why this occurred.

Heart rate 10 and 20 minutes post exercise

Schisandra supplementation of horses had a definite positive effect on lowering heart rate values at 10 and 20 minutes post exercise (pooled results). Lower post-exercise heart rates indicated that the horses had a faster recovery rate post exercise compared to horses in the control and CLA treatment groups. A quick post exercise recovery is very important in endurance racing, as a pre-determined heart rate has to be achieved by the horses during a race in order for them to pass the veterinary checks and continue in the race.

Respiration rate 0 minutes post exercise

The combination of the CLA and Schisandra (combination group) seemed to have increased the respiration rate at 0 minutes post exercise above that of horses supplemented only with one of the supplements fed on their own or those that received the control supplement (placebo). All the horses which received a supplement (CLA, Schisandra or combination) displayed higher respiration rates at 0 minutes post exercise compared to the control group, although not all of these were significant at the 95% level of significance. It has been known that Schisandra stimulates respiration by improving gas exchange in the lungs and the cells

of the body (Konstantinov, 1959), however the mechanism is unclear. The increase in respiration rate observed in the CLA group was most probably due to the stimulating effect of CLA on the metabolic rate (Kamphuis *et al*., 2003).

Respiration rate 10 minutes post exercise

An increase in respiration was observed in those horses that received either CLA, Schisandra or combination supplements in comparison to the control group; although, only CLA and Schisandra groups showed significant (P <0.05) increases in respiration rate 10 minutes post exercise.

Respiration rate 20 minutes post exercise

Again, a significant increased respiration rate was observed in the groups that received dietary supplements (CLA, Schisandra or combination) compared to the control group at 20 minutes post exercise. The CLA, Schisandra and combination supplements fed to the horses increased their respiration rates at 0, 10 and 20 minutes after exercise (not all significantly so). It is possible that this was due to an increased metabolic rate brought about by the different supplements. It has been shown in previous studies that CLA stimulates metabolism (Kamphuis *et al*., 2003), resulting in an increased metabolic rate. Moreover, *Schisandra chinensis* has been reported to stimulate respiration (Konstantinov, 1959), increasing oxygen intake and uptake and improving gas exchange in the lungs and the cells of the body.

Erythrocyte count

CLA significantly increased erythrocyte concentrations above that of the control and Schisandra groups. It is clear that CLA had a definite positive effect on erythrocyte concentrations. This could be a result of splenic contractions (resulting in the release of stored erythrocytes into bloodstream) possibly stimulated by CLA supplementation. This aspect needs to be investigated further with regards to ideal number of doses, best concentration to supplement as well as how long before race should supplement be fed to endurance horses.

Haemoglobin (Hgb)

From these results it is clear that CLA (fed alone or in combination with Schisandra) directly stimulates blood haemoglobin production, which in turn affects the erythrocyte concentration. Increased haemoglobin concentrations in the blood would result in increased performance, as more oxygen would reach the muscles, thus increasing the muscle capacity to do aerobic work. This was also reported by Lupandin *et al*. (1986). Furthermore, if more oxygen reaches the muscles, it would lead to a decrease in anaerobic respiration, which would result in less lactic acid production.

Leukocyte count

Horses in the CLA group had the highest leukocyte concentration, followed by the control, and then the combination treatment group. Horses in the Schisandra treatment group had the lowest leukocyte concentration. The only significant difference in terms of leukocyte concentration was between the CLA and Schisandra treatment groups. Multiple factors influence leukocyte production and concentrations; these results should thus be interpreted with caution.

Lactate Dehydrogenase (LDH)

CLA, Schisandra and combination treatment groups showed an increase in the enzyme concentrations above the control group (although the increase in the Schisandra group was not significant), as well as the reference range. This increase was especially apparent (and significant) in the groups that received dietary CLA supplements (CLA group and the combination group). From this it can be concluded that CLA increased the LDH concentrations in blood. LDH is an enzyme responsible for clearing lactic acid from the body. Horses with higher LDH concentrations in their blood would be able to clear lactic acid from their system more effectively, which could improve performance, as well as aid in the recovery of the body after strenuous exercise.

Blood urea nitrogen (BUN)

The lower BUN values observed in the combination group (compared to the CLA group) suggest improved digestion of protein and/or enhanced renal function. As CLA was present in the supplements of both the CLA group and combination group, CLA cannot be responsible for the lower BUN values in the combination group. It could be that Schisandra (present in the combination group) is responsible for these lower BUN concentrations in blood, as the BUN level in the Schisandra group was also lower than that of both the control and CLA groups, although not significantly so.

Total cholesterol

It seems as if the combination of CLA and Schisandra had an additive effect in terms of increasing cholesterol concentrations in horses, as neither the CLA nor the Schisandra increased cholesterol level significantly above the control group when supplemented separately. This is possibly due to the increased amounts of energy substrates made available to the body by the different supplements.

High density lipoprotein (HDL)

The large increase in total cholesterol observed in the combination group (above) is possibly due to the significant increase in HDL in the combination group above that of any other treatment group. As HDL is

regarded as “good cholesterol”, important for hormone synthesis and energy provision, this is a positive side effect.

Glucose

The lower glucose level in the CLA group, compared to the control group, could indicate a better uptake (and use) of glucose from the blood due to an increased rate of metabolism brought about by the CLA supplement (Kamphuis *et al*., 2003). CLA could have reduced insulin insensitivity, which would have resulted in an improved uptake of glucose from the blood. As CLA is known to stimulate mobilization of adipose tissue, the release of energy substrates from fat breakdown into the bloodstream should match the increased uptake of glucose. This effect should be investigated further in horses as well as other animal species (e.g. dogs) and humans. Feeding CLA to insulin insensitive horses could also yield favourable results, and this effect should be investigated.

Thyroxin (T4)

The combination of CLA and Schisandra (combination treatment group) had a significant effect on lowering blood T4 concentrations compared to the control and Schisandra treatment groups. It is possible that the combination of CLA and Schisandra stimulates the conversion of T4 (physiologically less active) to T3 (physiologically more active), in order to increase metabolic rate.

5.1.2 Summary of the treatment phase results

Basal heart rate

The lower basal heart rate of horses in the Schisandra and combination treatment groups in comparison to the CLA and control group indicates that Schisandra (supplemented on its own or in combination with CLA) has a significant effect on lowering the basal heart rate in horses.

Heart rate 10 minutes post exercise

Schisandra also had a clear effect on lowering heart rate values. As with the pooled results, the addition of CLA to the Schisandra supplements (as in the combination treatment group) reduced the heart rate lowering effect of Schisandra (the combination treatment group did not differ significantly from the control group). This could be due to the fact that CLA increases metabolism, which results in an increased heart rate.

Heart rate 20 minutes post exercise

CLA slightly decreased heart rate and the Schisandra constantly decreased heart rate. In the combination treatment group, the heart rate is lower than the control group (not significantly so), but higher than that of the Schisandra group (again, not significantly so).

Respiration rate 0 minutes post exercise

The combination of the CLA and Schisandra (combination group) seemed to have increased the respiration rate above that which each supplement would have if supplemented separately. Horses in the Schisandra and combination treatment groups displayed significantly higher respiration rates compared to the control group, although no significant difference was found between the CLA and control group.

Respiration rate 10 minutes post exercise

CLA supplementation significantly increased respiration rate in comparison to the control group. An increased metabolism brought on by CLA would result in the increased respiration. The Schisandra group also had higher respiration rates compared to the control group, although this was not significant. It is interesting to note that the combination group had lower respiration rates than both the CLA and Schisandra groups, but slightly higher than the control group (though, none of these differences were significant).

Respiration rate 20 minutes post exercise

Again an increased respiration rate was observed in the groups that received supplements (CLA, Schisandra or combination). This is probably due to an increased metabolic rate brought on by the different supplements.

Erythrocyte count

The control group had the lowest erythrocyte count (this effect was significant), while horses in the CLA group had a significantly higher erythrocyte count than horses in the Schisandra group. The combination treatment group also showed a significantly higher erythrocyte count than the control group. As Schisandra supplementation did not significantly increase the erythrocyte count above that of the control group, the increased erythrocyte count in the CLA and combination groups can thus only be as a result of CLA supplementation.

Haemoglobin (Hgb)

The CLA and combination treatment groups had significantly higher Hgb concentrations than the control group. These results confirm the haemoglobin generating effect of CLA, which also affects the erythrocyte concentrations.

Leukocyte count

As in the pooled results, horses in the CLA group had the highest leukocyte concentration, followed by the control, and then the combination treatment group. The Schisandra treatment group once again had the lowest leukocyte concentration. The only significant difference in terms of leukocyte concentration was between the CLA and Schisandra treatment groups. Multiple factors influence leukocyte production and concentrations; these results should therefore be interpreted with caution.

Lactate Dehydrogenase (LDH)

Horses which received dietary supplements (CLA, Schisandra and combination supplements) displayed an increase in LDH enzyme above the control group (although the increase in the Schisandra treatment group was not significant). Both the Schisandra and CLA stimulated LDH production, the CLA more so than the Schisandra, but the combination of the two supplements increased the LDH concentration the most.

Total cholesterol

CLA and Schisandra had an additive effect in increasing cholesterol concentrations in horses, as neither the CLA nor the Schisandra increased cholesterol level significantly above the control group when supplemented separately. This is possibly due to the increased amounts of energy substrates made available to the body by the combination of the two supplements.

High density lipoprotein (HDL)

Similar to the pooled results, the combination of CLA and Schisandra had an additive effect in increasing HDL concentrations in horses, as neither the CLA nor the Schisandra increased the cholesterol level significantly above the control group when supplemented separately.

Thyroxin (T4)

Similar to the pooled results, the combination of CLA and Schisandra (combination treatment group) had a significant effect on lowering blood T4 concentrations compared to the control and Schisandra treatment groups. It could be that the combination of CLA and Schisandra stimulates the conversion of T4 (physiologically less active) to T3 (physiologically more active), in order to increase metabolic rate.

5.1.3 Summary of the results for specific days

Day 0:

Basal heart rate

Horses in the Schisandra treatment group had the lowest basal heart rate, followed by the combination, and then the CLA treatment group. Horses in the control group had the highest basal heart rate. The only significant difference was between the control and Schisandra treatment groups. As no supplements had been given to the horses yet, this effect is possibly due to differences in fitness between the groups of horses.

Respiration rate 0 minutes post exercise

The respiration rate at 0 minutes post exercise of horses in the CLA treatment group differed significantly from the combination treatment group, but not from the control group or the Schisandra treatment group on day 0. The control group differed significantly from the combination treatment group, as well as the Schisandra treatment group in terms of respiration rate, 0 minutes post exercise on day 0. The respiration rate of the combination treatment group did not differ significantly from the Schisandra treatment group directly after exercise on day 0.

This is probably due to differences in fitness levels between the horses; the control group had the fittest horses with the lowest respiration rates, then the CLA group, then the Schisandra group and the least fit was the combination group (on Day 0).

Respiration rate 10 minutes post exercise

The only significant difference in respiration rate at 10 minutes post exercise on day 0 was found between the control group and the combination group. Horses in the CLA and Schisandra groups did not differ significantly from each other or the other two groups in terms of respiration rate, 10 minutes post exercise on day 0. The control group had the lowest respiration rate at 10 minutes post exercise, followed by the CLA then the Schisandra treatment groups. The combination treatment group had the highest respiration rate 10 minutes post exercise. Again this is possibly due to differences in fitness levels between the groups of horses.

Respiration rate 20 minutes post exercise

The CLA treatment group differed significantly from the control group in terms of respiration rate 20 minutes post exercise on day 0. However, the respiration rate of Schisandra and combination treatment groups did not differ significantly from each other or any other group at 20 minutes post exercise on day 0. The control group had the lowest respiration rate at 20 minutes post exercise, followed by the Schisandra then the combination treatment group. CLA treatment group had the highest respiration rate at 20 minutes post exercise.

Day 5:

No significant differences were found between the four groups of horses on day 5, indicating that all the horses reached the same level of fitness.

Day 10:

Respiration rate 20 minutes post exercise

The horses in the control group had the lowest respiration rate at 20 minutes post exercise on day 10, followed by the Schisandra group, then combination treatment groups. The CLA treatment group had the highest respiration rate, 20 minutes post exercise. Both the CLA and combination treatment groups differed significantly from the control group (but not from each other) in terms of respiration rate, 20 minutes post exercise on day 10. The Schisandra treatment group did not differ significantly from any other group with regards to respiration rate, 20 minutes post exercise on day 10.

The single dose of CLA had an effect on increasing respiration rate 20 minutes after exercise (when supplemented separately or in combination with Schisandra). This could be a result of a higher metabolism brought about by CLA supplementation.

Day 11:

Blood pH

When supplemented separately, CLA and Schisandra supplementation marginally increased the blood pH above that of the control group. The increase is very small and thus neglible. This is a once-off effect, as no significant differences were observed between the groups in the pooled and treatment phase results. There were also no significant differences in blood pH between the groups on day 19.

Erythrocyte count

CLA supplementation for only 2 days stimulated splenic contractions, resulting in a release of erythrocytes into the blood stream (when used as a supplement on its own or with Schisandra) on day 11. Increased erythrocyte in the blood would result in increased performance, as more oxygen would reach the muscles, increasing the muscle capacity to do aerobic work. Anaerobic respiration would also decrease if more oxygen reached the muscles, resulting in less lactic acid production.

Haemoglobin (Hgb)

The significantly higher blood Hgb concentration of horses in the CLA group, compared to that of the control group, suggests that CLA stimulated splenic contractions after only2 doses. This resulted in a release of erythrocytes into the blood stream, which increased the haemoglobin concentrations in the blood. Increased haemoglobin concentrations in the blood would result in increased performance, as more oxygen would reach the muscles, increasing the muscle capacity to do aerobic work. Increased haemoglobin concentrations in the blood would result in increased performance, as more oxygen would reach the muscles, increasing the muscle capacity to do aerobic work. This corresponds with the findings of Lupandin *et al*. (1986). Furthermore, anaerobic respiration would decrease if more oxygen reached the muscles, resulting in less lactic acid production.

Leukocyte count

As observed in both the pooled and treatment phase results, horses in the CLA group had the highest leukocyte concentration, followed by the control, and then the combination treatment group. The Schisandra treatment group once again had the lowest leukocyte concentration. The only significant difference in terms of leukocyte concentration was between the CLA and Schisandra treatment groups. Multiple factors influence leukocyte production and concentrations; these results should therefore be interpreted with caution.

Lactate dehydrogenase (LDH)

Horses in both the CLA and Schisandra treatment groups had higher LDH values on day 11 compared to the control group, although neither was significant. The combination group had significantly higher LDH values than the control group, which suggests that the combination of Schisandra and CLA stimulates LDH production. LDH is an enzyme responsible for clearing lactic acid from the body. Horses with higher LDH concentrations in their blood would be able to clear lactic acid from their system more effectively, which could improve performance, as well as aid in the recovery of the body after strenuous exercise.

High density lipoprotein (HDL)

Horses in the combination group displayed significantly higher HDL concentrations than any other group on day 11. This is probably due to the increased amounts of energy substrates made available to the body by the combination of CLA and Schisandra supplements.

Glucose

The lower glucose concentration of horses in the CLA and combination groups, compared to the control and Schisandra group, could indicate a better uptake (and use) of glucose from the blood, due to an increased rate of metabolism (brought on by the CLA supplement). CLA could have reduced insulin insensitivity, which

would have resulted in an increased uptake of glucose from the blood. This increased uptake and utilization of glucose could result in increased performance, as more energy is available to the muscles. . CLA's effect on insulin insensitivity should be investigated further in horses as well as other animal species (e.g. dogs) and humans.

Day 15:

Basal heart Rate

Horses in the CLA treatment group displayed increased basal heart rate values, but this was significant only when compared to the Schisandra treatment group. This could be due to a higher metabolism brought on by CLA supplementation. Schisandra had an effect on lowering the basal heart rate (both when supplemented alone or in combination with CLA) when compared to the control group, but this was not significant. The effects of these two supplements (CLA and Schisandra) are opposite, with CLA increasing basal heart rate, and Schisandra decreasing basal heart rate (neither was significant when compared to the control group). Because these two supplements have opposite effects on heart rates, it is not desirable for them to be used together.

Day 19:

Basal respiration rate

Basal respiration rate of horses in the combination group was significantly higher compared that of horses in the three other treatment groups on day 19. The higher basal respiration rate was possibly due to a higher metabolic rate brought on by the dietary supplements. A higher basal respiration rate would result in more oxygen reaching the muscles, and therefore less lactic acid production. Lower lactic acid concentration in muscles would increase the ability of muscles to perform work.

Heart rate 20 minutes post exercise

Schisandra, when supplemented on its own, drastically reduced the heart rate values compared to horses in the control group at 20 minutes post exercise. The other two treatment groups (CLA and combination) displayed lower heart rate values compared to the control group, but this was not significant.

Lactate Dehydrogenase (LDH)

The horses in the combination group displayed the highest LDH concentration, significantly higher than that of the control group. The horses in the CLA and Schisandra groups displayed higher LDH values compared

to the control group, but not significantly so. This suggests that the combination of Schisandra and CLA stimulates LDH production. LDH is an enzyme responsible for clearing lactic acid from the body. Horses with higher LDH concentration in their blood would be able to clear lactic acid from their system more effectively, which could improve performance, as well as aid in the recovery of the body after strenuous exercise.

High density lipoprotein (HDL)

CLA supplementation increased the HDL cholesterol concentration, significantly so when fed together with Schisandra (as in the combination group) compared to the control and Schisandra treatment groups.

It is interesting to note that no significant differences were found in terms of total cholesterol values on Day 11 or 19. The variation observed between the four groups regarding LDL concentration had also stabilized, as the LDL values are more similar on Day 19 compared to Day 0 and Day 11.

5.1.4 Summary of comparisons between initial (Day 0) and end (Day 19) physiological parameters in each group

Comparisons of treatment effects between day 0 and day 19 suggest that Schisandra had the most prominent effect on lowering respiration rates, both basal and post exercise. CLA's most prominent effect was the opposite, by increasing both basal and post exercise respiration rates. As these two supplements have opposite effects on respiration rate in horses, it is the opinion of the researchers that these two supplements should not be fed together.

5.2 Conclusion

Schisandra chinensis

The pooled results indicate that Schisandra had the most definite effect on lowering pre-exercise basal heart rate values, as well as lowering post exercise heart rate values.

In the pooled results, as well as the treatment phase, Schisandra had a leukocyte lowering effect, although this was not found in the blood results of any specific day. The horses in this study were in good health, and within the scope of the present study the supplements suggest an improvement in immune response. Schisandra also lowered respiration rates (basal and post exercise) from start to end of trial. Comparisons of treatment effects between day 0 and day 19 suggest that Schisandra had the most prominent effect on lowering respiration rates, both basal and post exercise.

CLA

In the pooled results the most significant effects of CLA supplementation were:

- Increased respiration rates most likely due to increased metabolic rate as a result of CLA supplementation. Increased respiration rate post exercise will result in faster recovery, however this may have a negative impact at the veterinary checks, as respiration rates are recorded.

- Decreased blood glucose as a result of increased uptake of glucose from the blood, as CLA supplementation is known to decrease insulin insensitivity. This could have resulted in an improved uptake of glucose from the blood. As CLA is known to stimulate mobilization of adipose tissue, the release of energy substrates from fat breakdown into the bloodstream should match the increased uptake of glucose. This effect should be investigated further in horses as well as other animal species (e.g. dogs) and humans. It would be interesting to evaluate the effect of CLA on insulin insensitive horses, however this is beyond the scope of this study.

- Increased erythrocyte and haemoglobin concentrations most likely due to splenic contractions (release of stored erythrocytes from splenic pool into circulatory system) probably stimulated by CLA supplementation.

- Increased LDH (lactate dehydrogenase) concentrations resulting in an increased ability of the body to clear lactic acid from the system.

In the treatment phase, the most significant effect of CLA supplementation was increased post exercise respiration rate. This is probably due to increased metabolic rate brought about by CLA supplementation.

On day 11, after one day of supplementation, the CLA group displayed higher erythrocyte and haemoglobin concentrations, and lower blood glucose concentrations. This increased erythrocyte and haemoglobin is most likely due to splenic contractions, which results in the release of stored erythrocytes into the blood stream, stimulated by CLA supplementation. The lower blood glucose concentration observed in the CLA group is thought to be a result of increased uptake of glucose into the cells of the body due to decreased insulin insensitivity.

Comparisons of treatment effects between day 0 and day 19 suggested that CLA supplementation increased both basal and post exercise respiration rates. As CLA and Schisandra supplementation had opposite effects on respiration rate in horses, the present results suggest that the supplements should not be fed together.

Combination

Pooled results:

The combination group had the highest post exercise respiration rates. This is most likely the result of an increased metabolic rate brought about by the CLA and Schisandra supplementation, which would lead to faster recovery. As respiration rates are checked at the veterinary gates, this could penalise the horse. More studies should be performed in real world conditions in order to evaluate this effect of increased respiration rate post exercise.

The combination group had significantly higher total cholesterol as well as HDL cholesterol values compared to the control group. This could be due to increased available energy substrates in the body as a result of CLA and Schisandra supplementation.

T4 concentration in the blood of the combination group was decreased. It could be that the combination of CLA and Schisandra stimulates the conversion of T4 (physiologically less active) to T3 (physiologically more active), in order to increase metabolic rate.

Treatment phase:

As in the pooled results, increased cholesterol and HDL values and decreased T4 concentrations were observed. Increased LDH (lactate dehydrogenase) concentrations of the combination group indicate an increased ability of these horses to break down lactic acid as a result of CLA and Schisandra supplementation.

On day 11 the LDH concentration is again elevated, as is the HDL cholesterol.

On day 19 the LDH concentration is still elevated, as is the HDL cholesterol. Average basal respiration rate of horses in the combination group is elevated, which is possibly due to an increase in metabolic rate brought on by CLA and Schisandra supplementation. At conclusion of the study all the horses were in good body condition and health. No supplemental feed was required in order to maintain body condition.

As rectal temperatures did not increase significantly during this study, it is the opinion of the researchers that these supplements did not place additional strain on these horses as a result of increased metabolism. Rather, it appears that the metabolic effects of the supplements were more on efficiency of respiration and energy metabolism.

Chapter 6

Critical evaluation

- Further studies are required to confirm these results.
- The number of horses used in the study should be increased, which may have improved the statistical significance and accuracy of the study.
- Bodyweights and body composition changes should also be monitored in order to evaluate the effect of CLA supplementation on bodyweight and composition of the horses.
- The exercise intensity should also be increased in order to evaluate the effectiveness of these supplements under more strenuous training conditions.
- These supplements should be fed to horses before an endurance race, in order to evaluate the effectiveness of these products under more realistic and stressful conditions.
- Different dosages of these supplements could also be given to the horses in order to determine the optimum dose.

References

Ahumada, F., Hermosilla, J., Hola, R., Pena, R., Wittwer, F., Wegmann, E., Hancke, J. & Wikman, G., 1989. Studies on the effect of *Schizandra chinensis* extract on horses submitted to exercise and maximum effort. Phytotherapy Research, Vol. 3, Issue 5, pp 175-179.

Akahoshi, A., Koba, K., Ohkura-Kaku, S., Kaneda, N., Goto, C., Sano, H., Iwata, T., Yamauchi, Y., Tsutsumi, K & Sugano, M., 2003. Metabolic effects of dietary conjugated linoleic acid (CLA) isomers in rats. Nutrition research 23 (2003) 1691-1701.

Argenzio R. A., Lowe J. E., Pickard D. W. & Stevens C. E., 1974. Digesta passage and water exchange in the equine large intestine. Am. J. Physiol. 226, 1035-1042.

Argenzio R. A., Southworth M., Lowe J. E. & Stevens C. E., 1977. Interrelationship of Na, HCO and volatile fatty acid transport by equine large intestine. Am. J. Physiol. 2, EX69-E478.

Argenzio, R. A., 1975. Functions of the equine large intestine and their interrelationship in disease. Cornell Vet. 65, 303-329.

Azain M.J., Hausman D.B., Sisk M.B., Flatt W.P., Jewell D.E. 2000. Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. J. Nutr., 130, 1548-1554.

Bailey, S.R., Rycroft, A. & Elliott, J., 2002. Production of amines in equine cecal contents in an in vitro model of carbohydrate overload. J. Anim. Sci. 80, 2656–2662.

Banni, S., Heys, S. D., & Wahle, K. W. J., 2003. Conjugated linoleic acids as anticancer nutrients: studies in vivo and cellular mechanisms. In J. - L. Sebedio, W. W. Christie, & R. Adlof (Eds.). Advances in conjugated linoleic acid research (Vol. 2, pp. 267–282). Champaign, IL: AOCS Press.

Belevych A.E., Beck R., Tammaro P., Poston L. & Smirnov S.V., 2002. Developmental changes in the functional characteristics and expression of voltage-gated K+ channel currents in rat aortic myocytes. Cardiovasc Res 54:152–161.

Belonosov, I.S. & Makarevich, N.I., 1958. *Schizandra chinensis* effect on carbohydratephosphoric metabolism. In: Belikov, I.F., Brekhman, I.I., Bykov, V.T., Lazarev, N.V., Serebryannik, B.E., Sorokhtin, G.N. (Eds.), Materials for the Study of Ginseng and Schizandra Roots. Far East Branch of USSR Academy of Science, Leningrad, pp. 159–165.

Bennett, D.K., 1980. Stripes do not make a zebra. Part 1. A cladistic analysis. Syst. Zool. 47, 272–287.

Bergero, D., Assenza, A. & Caola, G., 2005. Contribution to our knowledge of the physiology and metabolism of endurance horses. Livestock Production Science 92 (2005) 167–176.

Bretillon, L., Chardigny, J.M., Gregiore, S., Berdeaux, O., Sebedio, J.L., 1999. Effects of conjugated linoleic acid isomers on the hepatic microsomal desaturation activities in vitro. Lipids 34, 965–969.

Brown, K.M., 1987. Nutrient digestion in various segments of the gastrointestinal tract of ponies fed two, three or four meals per day. M.S. Thesis, Texas A&M University.

Burger, D., Dollinger, S., 1998. Raisons d'e´limination, etat de sante´et carrie`re sportive des chevaux dans les raids d'endurance en Europe et dans les pays arabes: approche statistique. Prat. Vet. Equine 30, 91 – 97.

Carlson G. P., 1987. Hematology and body fluids in the equine athlete: a review. Equine Exercise Physiology 2. Proceedings of the Second International Conference on Equine Exercise Physiology, San Diego, California Aug. 7-11 1986 (Edited by Gillespie J. R. and Robinson N. E.), pp. 393-424. ICEEP Publications, Davis, CA.

Castejon, F., Rubio, D., Tovar, P., Vinuesa, M. & Riber, C., 1994. A comparative study of aerobic capacity and fitness in three different horse breeds (Andalusian, Arabian and Anglo-Arabian). Zentralbl. Veterinarmed. 41, 645–652.

Chin, S.F., Storkson, J.M., Liu, W., Albright, K.J. & Pariza, M.W., 1994. Conjugated linoleic acid (9,11 and 10,12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. The journal of Nutrition, 124, 694-701.

Choi, Y.J., Kim, Y.C., Han, Y.B., Park, Y., Pariza, M.W., Ntambi, J.M., 2000. The trans-10, cis-12 isomer of conjugated linoleic acid downregulates stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes. J. Nutr. 130, 1920–1924.

Chrubasik, S. Pollak, A. Black, S., 2002. Effectiveness of devil's claw for osteoarthritis Rheumatology, 41 (2002), pp. 1332–1333.

Cook, M.E., Jerome, D.L., Crenshaw, T.D., Buege, D.R., Pariza, M.W., Albright, K.J., *et al.*, 1998. Feeding conjugated linoleic acid improves feed efficiency and reduces carcass fat in pigs. FASEB journal, 12, A836.

Cook, M.E., Miller, C.C., Park, Y. & Pariza, M., 1993. Immune modulation by altered nutrient metabolism-nutritional control of immune-induced growth depression. Poultry Science, 72, 1301-1305.

Corino, C., Mourot, J., Magni, S., Pastorelli, G., & Rosi, F., 2002. Influence of dietary conjugated linoleic acid on growth, meat quality, lipogenesis, plasma leptin and physiological variables of lipid metabolism in rabbits. Journal of Animal Science, 80, 1020–1028.

Corino, C., Pastorelli, G., Rosi, F., Bontempo, V., Rossi, R., 2009. Effect of dietary conjugated linoleic acid supplementation in sows on performance and immunoglobulin concentration in piglets. J. Anim. Sci. 87, 2299–2305.

d'Andrade F., 1979. Bref historique du cheval espagnol. In Special Andalou, Plaisirs Equestres, Crepin-Leblond, Paris, pp. 12-14.

Davies, Z., 2009. Introduction to Horse Nutrition, 1st ed., Wiley-Blackwell, Oxford, 2009, ISBN9781405169981, 248 pp

de Blomac, N. & Bogros, D., 1978. L'Arabe, premier cheval de Sang. Cropin-Leblond Editeur Paris.

de Deckere E.A., Van Amelsvoort J.M., Mcneill G.P., Jones P., 1999. Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster. British Journal of Nutrition, 82, 309-317.

De Fombelle A., Varloud M., Goachet A.G., Jacotot E., Philippeau C., Drogoul C., and Julliand V., 2003. Characterisation of microbial and biochemical profile along the anatomic parts of the digestive tract of horses fed two distinct diets. Anim. Sci., 77:293-304.

de Veth, M.J., Griinari, J.M., Pfeiffer, A.M., Bauman, D.E., 2004. Effect of CLA on milk fat synthesis in dairy cows: comparison of inhibition by methyl esters and free fatty acids and relationships among studies. Lipids 39, 365–372.

Denny M.J.S. & Dawson T. J., 1975. Effects of dehydration on body-water distribution in desert kangaroos. Am. J. Physiol. 229, 251-254.

Detweiler D.K. and Erickson H.H., Regulation of the Heart, in Dukes' Physiology of Domestic Animals, 12th ed., Reece W.O., Ed. Copyright 2004 by Cornell University.

Dugan, M.E.R., Aalhus, J. L., Schaefer, A. L., & Kramer, J. K. G., 1997. The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. Canadian Journal of Animal Science, 77, 723–725.

Duren, S.E., 2000. Feeding the endurance horse. In: Pagan, J.D. (Ed.), Advances in Equine Nutrition. Nottingham University Press, Nottingham, pp. 351–363.

Eaton, M.D., Hodgson, D.R., Evans, D.L., Bryden, W.L. & Rose, R.J., 1995. Effect of a diet containing supplementary fat on the capacity for high intensity exercise. Equine Exerc. Physiol. 4; Equine Vet. J. (Suppl. 18), 353– 356.

Edelman I. S., Liebman J., O'Meara M. P. & Birkenfeld L. W., 1958. Interrelationships between serum sodium concentration, serum osmolarity and total exchangeable sodium and total body water. J. Clin. Invest. 37, 1236-1256.

Ellis, A.D. & Hill, J., 2005. Nutritional Physiology of the Horse. Nottingham University Press, Nottingham, UK.

Erickson, H.H. & Poole, D.C., 2004. Exercise physiology. In: Dukes' Physiology of Domestic Animals, 12th ed. WO Reece (editor), Comstock Publishing.

Evans, D.L., 1994. The cardiovascular system: anatomy, physiology, and adaptations to exercise and training. In: Hodgson, D.R., Rose, R.J. (Eds.), The Athletic Horse. W.B. Saunders Co., Philadelphia, pp. 129–144.

Flaminio, M.J. & Rush, B.R., 1998. Fluid and electrolyte balance in endurance horses. Vet. Clin. North Am., Equine Pract. 14, 147– 158.

Foreman, J.H., 1998. The exhausted horse syndrome. Vet. Clin. North Am., Equine Pract. 14, 205– 219.

Frape, D.L., 1988. Dietary requirements and athletic performance of horses. Equine Vet. J. 20, 163– 172.

Garner, H.E., Coffman, J.R., Hahn, A.W., Hutcheson, D. & Tumbleson, M.E., 1975. Equine laminitis of alimentary origin: an experimental model. Am. J. Vet. Res. 36, 441–449.

Gaullier, J.M., Halse, J., Hoye, K., Kristianses, K., Fagertun, H., Vik, H., *et al.*, 2005. Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. The Journal of Nutrition, 135, pp778-784.

Geor, R. J., Harris, P. A.,Hoekstra, K. E., Pagan, J. D., 2001. Effect of corn oil on solid-phase gastric emptying in horses. J Vet Int Med 2001.

Goodson, J., Tyznik, W.J., Cline, J.H., & Dehority, B.A., 1988. Effects of an abrupt diet change from hay to concentrate on microbial numbers and physical environment in the caecum of the pony. Appl. Environ. Microbiol.54:1946–1950.

Griewe, K.M., Meacham, T.N., Fontenot, J.P., 1989. Effect of added dietary fat on exercising horses. Proc. 11th Equine Nutrition and Physiology Symposium ENPS USA, pp. 101– 106.

Griinari, J.M., Bauman, D.E., 2006. Milk fat depression: concepts, mechanisms and management. In: Sejrsen, K.T., Hvelplund, T., Nielson, M.O. (Eds.), Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 389–417.

Guyton A. C., 1986. Textbook of Medical Physiology. W. B. Saunders, PA.

Hancke, J., Burgos, R., Wikman, G., Ewertz, E. & Ahumada, F., 1994. *Schizandra chinensis*, a potential phytodrug for recovery of sport horses. Fitoterapia, Vol. 65, Issue 2, 1994, pp 113-118.

Harris, P. A., 1991. The Equine Rhabdomyolysis Syndrome in the United Kingdom: Epidemiological and clinical descriptive information. Br. vet. J. 147, 373

Harris, P.A. & Harris, R.C., 2005. Ergogenic potential of nutritional strategies and substances in the horse. Livestock Production Science 92 (2005) 147–165.

Harris, P.A., 1997. Energy sources and requirements of the exercising horse. Annu. Rev. Nutr. 17, 185–210.

Houseknecht, K. L., Vanden Heuvel, J. P., Moya-Camarena, S. Y., Portocarrero, C. P., Peck, L. W., Nickel, K. P., 1998. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the diabetic rat. In: Biochemical and Biophysical Research Communications, 244, 678–682.

Hughes, S.L., Potter, G.D., Greene, L.W., Odom, T.W. & Murray- Gerzik, M., 1995. Adaptation of Thoroughbred horses in training to a fat supplemented diet. Equine Exerc. Physiol. 4; Equine Vet. J. (Suppl. 18), 349– 352.

Huntington, P.J., Owens, E., Crandell, K., & Pagan, J., 2005. Nutritional management of mares – the foundation of a strong skeleton. In: Advances in Equine Nutrition. Nottingham University Press, Nottingham, UK.

Julliand, V., De Fombelle, A., & Varloud, M., 2006. Starch digestion in horses: The impact of feed processing. Livestock Science 100 (2006) 44–52.

Kaminski M. & De Andres Cara D.F., 1985. Electrophoretic markers of Andalusian Horses: Comparison of Spanish and Lusitanian lineages. Laboratoire d'Enzymologie, C.N.R.S., 91190 Gif-sur-Yvette, France.

Kaminski M. & Urbanska H., 1979. Electro-phoretic polymorphism of proteins in the blood of horses: studies of eleven pony breeds or populations. Biochem. Syst. Ecol. 7, 229 237.

Kaminski M., 1982. Genetic structure of populations of horses based on distribution of hemotypes. Biochem. Syst. Ecol. 10, 377-385.

Kamphuis, M.M.J.W., Lejeune, M.P.G.M., Saris, W.H.M. & Westerterp-Plantenga, M.S., 2003. The effects of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects. In: International Journal of Obesity 2003, 27, 840 - 847

Kienzle, E., Pohlenz, J. & Radicke, S., 1997. Morphology of starch digestion in the horse. Journal of Veterinary Medicine 44, 207–221.

Kienzle, E., Radicke, S., Landes, E., Kleffken, D., Illenseer, M. & Meyer, H., 1994. Activity of amylase in the gastrointestinal tract of the horse. Journal of Animal Physiology and Animal Nutrition 72, 234–241.

King J. M., 1979. Game domestication for animal production in Kenya: field studies of the body water turnover of game and livestock. J. Agric. Sci. Camb. 93, 71-79.

Konstantinov, A.A., 1959. Effect of *Schizandra chinensis* and Ginseng on tissue respiration. Transactions of the Khabarovsk Medical Institute 17, 63–64.

Kronfeld, D.S., 1996. Dietary fat affects heat production and other variables of equine performance under hot and humid conditions. Equine Vet. J. 11 (Suppl. 22), 24– 35.

Kronfeld, D.S., Ferrante, P.L., Taylor, L. & Custalow, S.W., 1995. Blood Hydrogen ion and lactate concentrations during strenuous exercise in the horse. Equine Vet. J. (Suppl. 18), 266–270.

Kuznetsova, T.F., 1958. On the pharmacology of Schizandra. Transactions of the Omsk Branch of the All-Union Society of Physiologists, Biochemists and Pharmacologists 1, 149–155.

Lapajev, I.I., 1978. Schizandra and its Curative Properties, 3rd ed. Khabarovskoye Knizhnoye Izdatelstvo (Russ), Khabarovsk, p. 48.

Latimer K.S., Mahaffey E.A., and Prasse K.W., Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 4th ed., Wiley-Blackwell, 2003.

Lauridsen C., Mu H., Henckel P., 2005. Influence of dietary conjugated linoleic acid (CLA) and age at slaughtering on performance, slaughter and meat quality, lipoproteins, and tissue deposition of CLA in barrows. Meat Science, 69, 393-399.

Lentz L.R., Valberg S.J., Herold L.V., Onan G.W., Mickelson J.R. & Gallant E.M., 2002. Myoplasmic calcium regulation in myotubes from horses with recurrent exertional rhabdomyolysis. Am J Vet Res 63:1724–31.

Lentz, L.R., Valberg, S.J., Balog, E.M., Mickelson, J.R. & Gallant, E.M., 1999. Abnormal regulation of muscle contraction in horses with recurrent exertional rhabdomyolysis. Am. J. Vet. Res. 60, 992–999.

Levin, N. C., Nelson, C., Gurney, A., Vandlen, R., & Sauvage, F., 1996. Decreased food intake does not completely account for adiposity reduction after protein infusion. Proceedings of the National Academic of Sciences of the United States of America, 93, 1726–1730.

Lucke, J. N., 1982. Proceedings of the Association of Veterinary Anaesthetists of Great Britain and Ireland 9, 140.

Lupandin, A.V. & Ovsyanikova, V.Y., 1986. Increasing resistance to unfavourable factors under the effect of *Schizandra chinensis* extract. In: Brekhman, I.I. (Ed.). Physiological Mechanisms of Adaptation. Ivanovo State University, Ivanovo, p.92–97.

MacLeay, J.M., Sorum, S.A., Valberg, S.J., Marsh, W.E. & Sorum, M.D., 1999. Epidemiologic analysis of factors influencing exertional rhabdomyolysis in Thoroughbreds. Am. J. Vet. Res. 60, 1562–1566.

Martín, D., Antequera, T., González, E., López-Bote, C.J., Ruíz, J., 2007. Changes in the fatty acid profile of the subcutaneous fat of swine throughout fattening as affected by dietary conjugated linoleic acid and monounsaturated fatty acids. J. Agric. Food Chem. 55, 10820–10826.

McGowan C.M., Fordham T. & Christley R.M., 2002. Incidence and risk factors for exertional rhabdomyolysis in Thoroughbred racehorses in the United Kingdom. Vet Rec. 151:623–6.

McKenzie E.C., Valberg S.J., Godden S.M., *et al.*, 2003a. Effect of dietary starch, fat, and bicarbonate content on exercise responses and serum creatine kinase activity in equine recurrent exertional rhabdomyolysis. J Vet Intern Med 2003a; 17:693–701.

McKenzie, E.C., Valberg, S. & Pagan, J.D., 2003b. Nutritional Management of Exertional Rhabdomyolysis. In: Robinson, N.E. (Ed.), Saunders, St Louis (MO).

Medina, B., Drogoul, C. & Julliand, V., 2002. Pratiques alimentaires et transit digestif. In: Les Haras Nationaux (Ed.), 28e`me Journe´e de la recherche e´quine. Les Haras Nationaux, Paris, pp. 91– 102.

Mlekoday, J.A., Mickelson, J.R., Valberg, S.J., Horton, J.H., Gallant, E.M. & Thompson, L.V., 2001. Calcium sensitivity of force production and myofibrillar ATPase activity in muscles from Thoroughbreds with recurrent exertional rhabdomyolysis. Am J Vet Res. 62:1647–52.

Morales, J., Gatnau, R., Pineiro, C., 2008. Conjugated linoleic acid and tryptophan supplementation improve immune response of weaned piglets. In: Joint Annual Meeting of American Society of Animal Science (ASAS), p. W-133.

Murray, M.J., Schusser, G.G., Pipers, F.S. & Gross, S.J., 1996. Factors associated with gastric lesions in Thoroughbred racehorses. Equine Vet. J. 28, 347–368.

National Research Council, 1989. Nutrient Requirements of Horses, 5th ed. revised. National Academy of Sciences, Washington, DC, USA.

Neumann, G., 1990. La struttura della prestazione negli sport di resistenza. Riv. Cult. Sportiva SdS 20, 66–77.

Newsholm, E.A., Blomstrad, E., Ekblom, B., 1992. Physical and mental fatigue: metabolic mechanisms and importance of plasma amino acids. Br. Med. Bull. 48, 477– 495.

Nobis G., 1974. The origin, domestication and early history of domestic horses. Vet. Med. Rev. 3, 211-225.

Orloff M. J. & Hutchin P., 1972. Fluid and electrolyte response to trauma and surgery. In Clinical disorders of Fluid and Electrolyte Metabolism. Clin. Sci. 72. 647-655.

Orme, C.E., Harris, R.C., Marlin, D.J. & Hurley, J.S., 1997. Metabolic adaptation to a fat supplemented diet in the Thoroughbred horse. Br. J. Nutr. 78, 443– 458.

Orsini, J.A., Wrigley, J. & Riley, P., 2010. Home Care for Horses with Chronic Laminitis. Veterinary Clinics of North America: Equine Practice. Volume 26, Issue 1, April 2010, Pages 215–223.

Ostrowska, E., Muralitharan, M., Cross, R. F., Baumann, D. E., & Dunshea, F. R., 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. Journal of Nutrition, 129, 2037–2042.

Pagan, J., D., 1998. Electrolytes and the performance horse. In: J. D. Pagan (Ed.) Advances in Equine Nutrition. P. 201-204. Nottingham University Press. Nottingham, United Kingdom.

Pariza, M. W., Park, Y., & Cook, M. E., 2001. The biologically active isomers of conjugated linoleic acid. Progress in Lipid Research, 40, 283–298.

Park, Y., Albreight, K. J., Liu, W., Storkson, J. M., Cook, M. E., & Pariza, M. W., 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids, 32, 853–858.

Patterson, R., Connor, M., Nyachoti, C.M., 2007. Effects of conjugated linoleic acid (CLA) on sow reproductive performance. J. Anim. Sci. 85 (suppl. 1), 507–508.

Pearson, W., McKee, S., Clarke, A.F., 1999. The effect of a proprietary herbal product on equine joint disease. Journal of Nutraceuticals, Functional and Medicinal Foods 2, 31–46.

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Boone, T., & Collins, F., 1995. Effect of obese gene product on body weight regulation in ob/ob mice. Science (Washington DC), 296,540–543.

Prince, A., Geor, R., Harris, P., Hoekstra, K., Gardner, S., Hudson, C. & Pagan, J., 2001. Comparison of the metabolic responses of trained Arabian and Thoroughbred horses during high and low intensity exercise. Proceedings 17th Symposium of the Equine Nutrition and Physiology Society, Lexington, Kentucky, May 31–June 2, 2001. , pp. 267– 272.

Reece W.O., Respiration in Mammals, in Dukes' Physiology of Domestic Animals, 12th ed., Reece W.O., Ed. Copyright 2004 by Cornell University.

Reeves, M.J., Salman, M.D. & Smith, G., 1996. Risk factors for equine acute abdominal disease (colic): results from a multi-center case control study. Prev. Vet. Med. 26, 285–301.

Robertshaw, D., Temperature Regulation and Thermal Environment, in Dukes' Physiology of Domestic Animals, 12th ed., Reece W.O., Ed. Copyright 2004 by Cornell University.

Robinson, N.E., Sprayberry, K.A., 2009. Therapy in Equine Medicine, sixth edition. Elsevier Health Sciences.

Rose, R.J., Arnold, K.S., Church, S. & Paris, R., 1980. Plasma and sweat electrolyte concentrations in the horse during long distance exercise. Equine Vet. J. 12, 19– 22.

Rowe, J.B., Lees, M.J. & Pethick, D.W., 1994. Prevention of acidosis and laminitis associated with grain feeding in horses. J. Nutr. 124, 2742S–2744S.

Ruckebusch Y., Phaneuf L. P. & Dunlop R., 1991. Physiology of Small and Large Animals. B. C. Decker, PA.

Ryke, P. A. J., 1987. Evolusie. Potchefstroom University, PhD Thesis.

Schott, H.C., McGlade, K.S., Molander, H.A., Leroux, A.J. & Hines, M.T., 1997. Body weight, fluid, electrolyte, and hormonal changes in horses competing in 50- and 100-mile endurance rides. Am. J. Vet. Res. 58, 303–309.

Sewell, D.A. & Harris, R.C., 1992. Adenine nucleotide degradation in the Thoroughbred horse with increasing exercise duration. Eur. J. Appl. Physiol. 65, 271– 277.

Sewell, D.A., Harris, R.C., Hanak, J. & Jahn, P., 1992. Muscle adenine nucleotide degradation in the Thoroughbred horse as a consequence of racing. Comp. Biochem. Physiol. 101B, 375– 381.

Shephard, R. J., 1982. Physiology and Biochemistry of Exercise. New York: Praeger.

Sisson, S. & Grossman J. D., 1953.The Anatomy of Domestic Animals. W. B. Saunders, PA.

Smith, S.B., Hively, T.S., Cortese, G.M., Han, J.J., Chung, K.Y., Castenada, P., Gilbert, C.D., Adams, V.L., Mersmann, H.J., 2002. Conjugated linoleic acid depresses the _9-desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. J. Anim. Sci. 80, 2110–2115.

Snow, D.H., Baxter, P. & Rose, R.J., 1981. Muscle fibre composition and glycogen depletion in horses competing in an endurance ride. Vet. Rec. 108, 374– 378.

Snow, D.H., Mason, D.K., Ricketts, S.W. & Douglas, T.A., 1983. Post-race blood biochemistry in Thoroughbreds. Equine exercise physiology. Proceedings of the First International Conference, pp. 389– 399.

Song, H.J., Sneddon, A.A., Barker, P.A., Bestwick, C., Choe, S.N., McClinton, S., *et al*., 2004. Conjugated linoleic acid inhibits proliferation and modulates protein kinase C isoforms in human prostate cancer cells. Nutrition and Cancer, 49, 100-108.

Sosa Leon, L.A., 1998. Treatment of exercise-induced dehydration. Vet. Clin. North Am., Equine Pract. 14, 159–173.

Spangfors, P., 2000. Some aspects of feeding the endurance horse. In: Pagan, J.D. (Ed.), Advances in Equine Nutrition. Nottingham University Press, Nottingham, pp. 341– 349.

Szymczyk, B., Pisulewski, P.M., Szczurek, W., Hanczakowski, P., 2001. Effects of conjugated linoleic acid on growth performance, feed conversion efficiency, and subsequent carcass quality in broiler chickens. Br. J. Nutr., 85, 465- 473.

Valberg, S., 2009. Exertional myopathies in horses. In: Smith, B.P. (Ed.), Large Animal Internal Medicine. Mosby, St Louis, p. 1414.

Wagner, H. & A. Proksch 1985. Immunostimulatory drugs of fungi and higher plants. IN: (H. Wagner *et al*. Eds.) Economic and medicinal plant research. Vol. 1. Academic Press, New York, New York. Pp.113-153.

Weineck, J., 1990. Manual d'Entrainement. Vigot, Paris, pp. 424.

Wiegand, B.R., Parrish Jr., F.C., Swan, J.E., Larsen, S.T., Baas, T.J., 2001. Conjugated linoleic acid improves feed efficiency, decreases subcutaneous fat and improves certain aspects of meat quality in stress-genotype pigs. J. Anim. Sci. 79, 2187–2195.