A Forage-Only Diet and Reduced High Intensity Training Distance in Standardbred Horses

Growth, Health and Performance

Sara Ringmark

Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management
Uppsala

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Abstract

This thesis examined growth, health and performance in sixteen 1.5-3-year-old Standardbred horses fed a forage-only diet and trained to race as 3-year-olds in either a control training programme (C-group) or a training programme in which the high intensity training distance was reduced by 30% (R-group). Body condition, height, body weight, exercise heart rate (HR), veterinary treatments, health status, locomotion symmetry and days lost to training were recorded continuously. Lactate, haematocrit and HR response to a standardised exercise were measured, as was lactate threshold (V_{La4}). A muscle biopsy was taken yearly for each horse and analysed for glycogen content. A complementary study examined the effect of forage crude protein (CP) content on insulin response to feeding in six adult, conditioned Standardbred horses.

All horses in the main study passed a preparation race, 94% qualified for races and 56% raced. Experimental horses raced to a similar extent and passed preparation and qualification races to a higher extent than their older siblings and horses of the same cohort. There was no difference between training groups with respect to race participation, post-exercise blood lactate or V_{La4}. R-group horses showed less pronounced cardiovascular response to training than C-group horses. There was no difference between the groups in clinical health examinations or locomotion asymmetry but R-group horses lost fewer days to training. Locomotion asymmetry in all horses increased after introduction of new speed training and high asymmetry was associated with later qualification for races. No cases of feed-related heath disorders occurred during normal management. The horses grew least as well as reported in the literature and body condition was maintained at a moderate level. Resting muscle glycogen content was within the range reported previously for concentrate-fed horses. In the study on adult horses, the CP content and the content of water-soluble carbohydrates (WSC) together explained more of the variation in insulin response to feeding than WSC content alone. It was concluded that a forage-only diet and reduced training distance can benefit health and poses no limitation to race participation, growth, body condition or muscle glycogen content in 3-year-old Standardbred horses.

Keywords: Forage-only diet, training distance, lactate threshold, heart rate, muscle glycogen, health status, locomotion symmetry, days lost to training.

Author's address: Sara Ringmark, SLU, Department of Animal Nutrition and Management, P.O. Box 7024 Uppsala, Sweden
E-mail: Sara.Ringmark@slu.se

Dedication

To all horses, for everything you give.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ringmark, S., Roepstorff, L., Essén-Gustavsson, B., Revold, T., Lindholm, A., Hedenström, U., Rundgren, M., Ögren, G. and Jansson, A. (2013). Growth, training response and health in Standardbred yearlings fed a forage-only diet. *Animal* 7(5), 746-753.

II Ringmark, S., Lindholm, A., Hedenström, U., Lindinger, M., Dahlborn, K., Kvart, C. and Jansson, A. Effects of high intensity training distance on V_{La4} and cardiovascular response of 2-3 year old Standardbred horses. (Submitted)

III Ringmark, S., Jansson, A., Lindholm, A., Hedenström, U. and Roepstorff, L. A 2.5 year study on health and locomotion symmetry in young Standardbred horses fed a forage-only diet and subjected to two levels of high intensity training distance. (Submitted)

IV Ringmark, S., Hedenström, U., Lindholm, A., Revold, T., Roepstorff, L. and Jansson, A. Effects of high intensity training distance on race participation, muscle glycogen and body condition in young Standardbred horses fed a forage-only diet. (Manuscript)

V Ringmark, S. and Jansson, A. (2013). Insulin response to feeding forage with varying crude protein and amino acid content in horses at rest and after exercise. *Comparative Exercise Physiology* 9(3-4), 209-217.

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Abbreviations

ATP	Adenosine tri-phosphate, energy molecule
AUC	Area under curve for insulin response
BCS	Body condition score
bpm	Beats per minute
BW	Body weight
CP	Crude protein
DM	Dry matter
DW	Dry weight
HR	Heart rate
HR10	Recovery HR 10 minutes post exercise
LVIDd	Left ventricular internal diameter during diastole
ME	Metabolisable energy
NDF	Neutral detergent fiber
V_{200}	Velocity at heart rate 200 beats per minute
VFA	Volatile fatty acids
V_{La4}	Anaerobic threshold, velocity at blood lactate concentration 4 mmol/L
VO_{2max}	Maximal aerobic capacity
VS	Vector sum calculated from maximum and minimum differences in head and pelvic sensors
WSC	Water soluble carbohydrates

1 Background

Sweden, France and the USA are regarded as being among the world's most successful nations in terms of Standardbred (harness) horse racing. In Sweden, about 3,000 Standardbred horses are born each year and 18,000 horses are registered at a trainer. The Swedish Standardbred horse is bred for competition at an early age and as 2-year-olds about 70% participate in a preparation race (*premielopp* in Swedish). However, despite an early race debut being the goal for most horses, only about 40-60% of each cohort in Sweden race at least once before four years of age (Swedish Trotting Association, 2014). It is known from previous studies that health problems are the most common reason for performance failure and training interruption in race horses (Rossdale *et al.*, 1985, Bendroth, 1986, Bailey *et al.*, 1999, Vigre *et al.*, 2002).

1.1 Feeding of racehorses

To reach the goal of competing as 2- or 3-year-olds, training of Standardbred horses starts already as 1-year-olds. Consequently, these horses are submitted to an increased training load while growth is still occurring and energy and nutrient intake requirements are correspondingly high. To meet the high energy requirements of racehorses, starch-rich feeds are commonly used (Jansson and Harris, 2013). However, feeding with starch-rich feeds has been associated with a number of health problems, such as colic (Tinker *et al.*, 1997b), rhabdomyolysis (Lindholm *et al.*, 1974b, MacLeay *et al.*, 1999), gastric ulcers (Luthersson *et al.*, 2009) and stereotypic behaviours (Redbo *et al.*, 1998). It is therefore important to find a feeding strategy that can lower these risks without impeding performance.

1.2 Training of racehorses

The training of racehorses is performed with two main aims in mind: to enhance performance capacity and to prevent injuries by adaptation of tissues to increased loads. The type of training to which racehorses are submitted is associated with improvement in aerobic metabolism (Evans and Rose, 1988, Roneus *et al.*, 1994) and alterations in locomotive tissues (Firth, 2006). High intensity exercise, like Standardbred horse training for races, causes heat and lactic acid production, increased extracellular potassium concentrations and depletion of muscle ATP and glycogen (Snow and Valberg, 1994). These are factors within the body system that must be stressed to some extent to improve aerobic capacity and performance, but they may also contribute to fatigue and overload of musculoskeletal tissues. When a horse becomes tired, the locomotion pattern and joint loads are altered (Johnston *et al.*, 1999) and the risk of mistakes leading to injury causing acute overloads increases. Both cumulative and acute overloads may cause lameness, which is reported to be the most common health problem in sport horses (Bendroth, 1986, Vigre *et al.*, 2002, Dyson *et al.*, 2008). The design of training programmes most likely has a great influence on both the physiological responses related to performance (Lindholm and Saltin, 1974) and the ability of the musculoskeletal tissues to adapt. However, no study to date has applied a long-term perspective to document the effects on performance-related physiological parameters and health of different training strategies from breaking to racing in Standardbred horses.

2 Introduction

2.1 Dietary energy substrates

Due to the high training intensity, Standardbred race horses have high energy requirements, amounting to about twice their requirements for maintenance. Energy-dense feedstuffs are therefore necessary and the diet of racehorses all over the world usually consists of approximately 4-11 kg concentrate and 3-10 kg forage (Jansson and Harris, 2013). However, the horse is by its evolution adapted to feed on forage, which requires grazing for 10-18 h per day (Duncan, 1991) and an ability to digest structural carbohydrates. Concentrates, on the other hand, are most often based on starch-rich cereals and are associated with a number of health problems, including altered cartilage development in growing horses (Glade and Belling, 1984). Feeding ≥2.5 kg/day of concentrates can increase the risk of colic five-fold (Tinker *et al.*, 1997b) and gastric ulcers are more common in horses fed high amounts of concentrates (Luthersson *et al.*, 2009).

Dietary energy may be derived from carbohydrates, fat or protein. Depending on the composition of the diet, different proportions of energy substrates will be available to the animal (Hintz *et al.*, 1971b) to use for maintenance, growth and exercise. The ingestion of soluble carbohydrates, such as starch, normally results in intestinal breakdown by pancreatic enzymes. Energy substrates in the form of simple sugars, such as glucose, are then absorbed through the intestinal wall (Fonnesbeck, 1969, Hintz *et al.*, 1971b) (Figure 1). If excessive amounts of starch (>2-4 g/kg body weight (BW) (Kienzle, 1994)) are ingested, leakage of starch into the hindgut may occur and cause rapid fermentation that produces lactic acid and lowers the pH (Julliand *et al.*, 2001, Medina *et al.*, 2002). In contrast, when structural carbohydrates or fibre compounds such as cellulose, hemicellulose and pectin are ingested, the main energy absorption does not occur until these reach the hindgut (colon and caecum) (Fonnesbeck, 1969, Hintz *et al.*, 1971b). The β-linkages in dietary fibre are resistant to the pancreatic enzymes in the small intestine, but can be broken by microbial fermentation. The hind gut of a horse hosts more than 10^8 microbes/g contents (Kern *et al.*, 1973), which mainly utilise dietary fibre as an energy substrate and produce volatile fatty acids (VFAs; *i.e.* acetic, propionic and butyric acid). The VFAs are absorbed through the intestinal wall (Argenzio *et al.*, 1974) and can be utilised for glucose production, fat synthesis and energy production (Figure 1). Like the VFAs, dietary fat can be utilised for energy production or energy storage in fat cells. The digestibility of oil in horses is high (>85% (McCann *et al.*, 1987, Kronfeld *et al.*, 2004)), and after emulsification by bile salts, dietary fat is broken down by pancreatic enzymes and absorbed through the intestinal wall. Protein generally makes a low contribution to the energy supply of the horse (Lawrence, 1990) but may after broken down to amino acids be absorbed mainly in the small intestine, but also to some extent in the hindgut (Hintz *et al.*, 1971b).

The proportions of energy substrates in feedstuffs commonly fed to horses vary greatly (Table 1). The composition of the diet thereby has the potential to affect the format of energy available to the horse. The composition of forage may also vary greatly with *e.g.* botanical stage at cut, species present, fertilisation, weather, soil type and season. Analyses of forages grown for horses during 2002 in a Swedish feed laboratory revealed a variation in content of metabolisable energy (ME) of 6-12.5 MJ/kg dry matter (DM) (Jansson *et al.*, 2011). By cutting the grass at an early botanical stage, it is possible to obtain high contents of energy that are comparable to, or higher than, those in cereal (Table 1). The development of anaerobic conservation methods for forage has contributed to less weather dependence during harvesting and has thereby increased the chances of harvesting forages at a maturity stage with adequate nutrient composition and energy content suitable for horses with high requirements.

Table 1. *Content of metabolisable energy (ME), fat, starch, water-soluble carbohydrates (WSC) and neutral detergent fibre (NDF) per kg dry matter in some common feedstuffs fed to horses. After Spörndly and Burstedt (2003) using equations in Jansson et al. (2011)*

Feed	ME, MJ	Fat, g	Starch, g	WSC, g	NDF, g
Oil	31.3	100	0	0	0
Oats	9.7	61	338	10	358
Barley	11.0	27	518	24	229
Maize	12.1	50	712	17	94
Molassed sugar beet pulp	11.3	23	0	242	334
Early cut grass forage[1]	11.2	20	0	110	550
Mid-season cut grass forage[1]	9.5	20	0	110	670

[1]Timothy, meadow fescue, ryegrass and cocksfoot.

Absorption of carbohydrates from the small intestine causes elevated levels of blood glucose, followed by increased levels of the anabolic hormone insulin. Insulin stimulates uptake and storage of glucose in liver, muscles and after conversion to fat also in fat tissue. Consumption of concentrates results in higher insulin levels than consumption of forage (Stull and Rodiek, 1995, Connysson *et al.*, 2010) and an intake of 0.9 kg finely milled maize can elevate plasma insulin levels from 4.3 to 29.1 μU/mL at 90 minutes post feeding (Vervuert *et al.*, 2004b). Through the insulin-mediated activation of glycogen synthase (Devlin and Horton, 1985), glucose is stored in the liver and muscle cells in the form of glycogen, which is one of the main energy sources used during prolonged or intensive muscle work. The muscle glycogen stores in the horse are large compared with those in man (Lindholm *et al.*, 1974a) and the glycogen levels in muscle tissue are usually around 500-650 mmol/kg dry weight (Valberg, 2014).

2.1.1 Energy substrates for growth

Growing horses at 6-24 months of age have 130-200% higher energy requirements for maintenance and growth than adult horses of the same BW has for maintenance (NRC, 2007). The energy substrates used to meet the requirements of growing horses could possibly affect their health as an adult. High amounts of starch-rich feeds have been suspected to increase the risk of abnormal cartilage development in growing horses (Glade and Belling, 1984). Insulin sensitivity, which if decreased is associated with metabolic syndrome (Frank *et al.*, 2010) and possibly also an increased risk to develop osteochondrosis in genetically predisposed horses (Ralston, 1996, Henson *et al.*, 1997), was found to be 37% lower in yearling horses fed a diet with 49% nonstructural carbohydrates from birth compared with yearlings fed a diet containing 12% nonstructural carbohydrates in a study by Treiber *et al.* (2005). It has even been indicated that feeding pregnant mares a diet containing 39% starch may tend to decrease insulin sensitivity and increase plasma glucose concentrations in the newborn foals up to at least 160 days of age, compared with foals with dams fed a diet containing only 4% starch (George *et al.*, 2009). In studies in which the starch in concentrates has been partly replaced by fat (10% of concentrate), growth rate has not been impaired compared with the controls (Saastamoinen *et al.*, 1994, Ott and Kivipelto, 1998, Ropp *et al.*, 2003). However, in another study by Ott *et al.* (2005), weanlings fed no starch but instead a diet with maize oil gained 17% less BW than the control group fed a diet containing 31% starch. In the same study, weanlings fed a diet with 17% starch showed similar weight gain to weanlings fed a diet containing 35% starch. This led the authors to suggest that growing horses require an easily digested source of carbohydrates, such as starch, for glucose production to obtain maximum growth rate. In contrast, Jansson *et al.* (2012) found no negative effect on weight gain in weanlings fed a forage-only diet comprised of a high-quality forage compared with in weanlings fed a traditional diet comprised of hay and concentrates. Besides energy intake, growth rate is also strongly dependent on adequate intake of crude protein (CP) and CP quality, particularly the lysine content (Saastamoinen and Koskinen, 1993).

Weanlings fed *ad libitum* may by an increased DM intake retain the same energy intake and body weight (BW) gain when fed a diet with a high (73-78%) inclusion of forage as when fed a diet composed of 62% concentrates and 33% of forage (Cymbaluk and Christison, 1989). In another study, Cymbaluk *et al.* (1989) compared growth in *ad libitum* fed weanlings with weanlings fed according to recommendations until 24 months of age. The *ad libitum* fed horses consumed 21-44% more digestible energy and gained 24% more BW and 11% more height than the horses fed according to recommendations.

2.2 Exercise energy metabolism

The energy utilised during muscular work may be derived from both aerobic and anaerobic pathways (Figure 1). In the presence of oxygen, ATP can be produced from oxidative phosphorylation of carbohydrates and fatty acids. In the beginning of muscular work, before oxidative pathways have begun to produce energy, anaerobic energy production occurs in the cell cytoplasm, mainly by the phosphocreatine reaction and the myokinase reaction. However, the most important pathway for anaerobic energy production is anaerobic glycolysis, which produces fewer ATP molecules than the aerobic pathways and also lactate, which can be used for energy production after hepatic conversion to glucose. The aerobic and anaerobic pathways both contribute to energy production during exercise, with the relative contributions varying with exercise intensity, exercise duration and substrate availability (Lindholm *et al.*, 1974a, Geor *et al.*, 2000a, Geor *et al.*, 2000b, Votion *et al.*, 2007, Pratt-Phillips and Lawrence, 2014). Consequently, fat, protein and carbohydrates are used in different combinations depending on availability and type of exercise, with more energy derived from fat during low intensity exercise and more energy derived from carbohydrates as the intensity increases (Votion *et al.*, 2007). Moreover, training causes a shift in substrate use towards more fat oxidation (Thomas *et al.*, 1983) and less glycogen use (Essen-Gustavsson *et al.*, 1989, Geor *et al.*, 2002), accompanied by increased amounts of oxidative enzymes in muscles (Essen-Gustavsson *et al.*, 1983, Roneus *et al.*, 1992), which promotes aerobic metabolism of substrates.

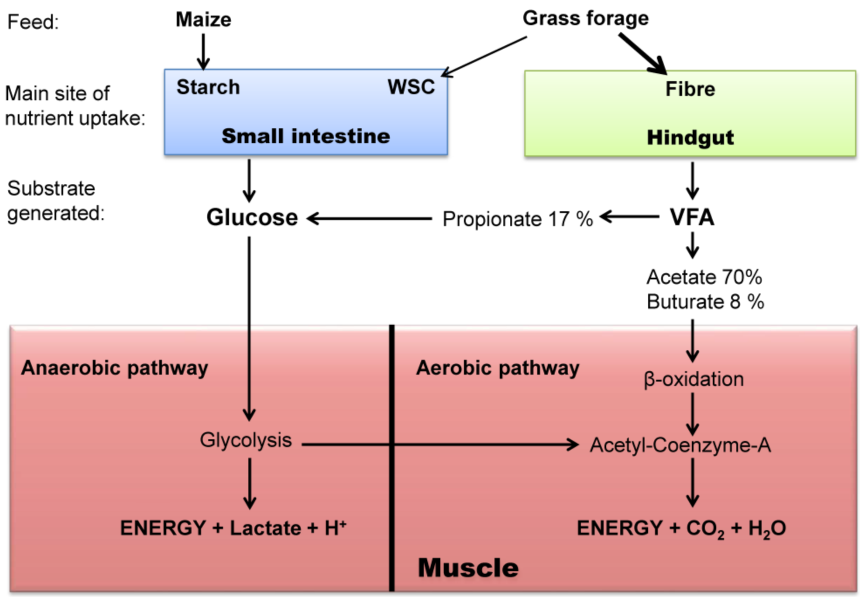

Figure 1. Simplification of the digestive and metabolic pathways used in energy utilisation from different types of carbohydrates in maize and forage. In the aerobic pathways, glycolysis and β-oxidation of fat generate acetyl-coenzyme-A, which through several steps in the citric acid cycle and electron transport chain generates ATP, carbon dioxide and water. Short-chain fatty acids, like VFAs, can enter the muscle cell freely, in contrast to long-chain fatty acids which first have to be esterified (Votion *et al.*, 2007). Butyrate is converted to acetate in the liver. Acetate is converted by β-oxidation to acetyl-coenzyme-A (Votion *et al.*, 2007). Propionate (~17% of VFA (Hintz *et al.*, 1971a, Argenzio *et al.*, 1974)) is converted to glucose through hepatic gluconeogenesis. The anaerobic combustion of glucose generates less ATP than when glucose is oxidised. Anaerobic glycolysis also generates lactate and hydrogen ions, which can cause a decrease in muscle cellular pH (Valberg, 2014).

During exercise, plasma insulin concentration decreases (Thornton, 1985) to facilitate mobilisation of substrates and glucose enterers the muscle cells without insulin-mediated transport. The importance of available glucose increases with exercise intensity and recruitment of a higher proportion of glycolytic muscle fibres (type II fibres). Glycogen stores provide most of the glucose required for energy production during intensive exercise, and regular exercise in combination with a low intake of carbohydrates may deplete glycogen stores (Topliff *et al.*, 1983). Performance may remain at constant levels in Thoroughbreds performing intensive exercise after a glycogen depletion of 22% (Davie *et al.*, 1996), but low levels (80% depletion) of muscular glycogen decrease runtime to fatigue by as much as 46% (from 130 to 71 seconds) (Lacombe *et al.*, 2001) and practices to spare glycogen during exercise are therefore desirable. Muscle glycogen utilisation is approximately three-fold greater at fast trot than at slow (5.0 vs. 8.3 m/s (Lindholm *et al.*, 1974a). However, time of work is often short during intensive exercise and 2400 m of trot, split into four intervals, at a speed of 12.5 m/s can deplete glycogen stores by ~50% (Lindholm *et al.*, 1974a). Following an exercise consisting of warm-up followed by 2600 m at ~90% of VO_{2max}, glycogen was depleted by approximately 30% in a study by Palmgren-Karlsson *et al.* (2002).

The glucose used during exercise may also be blood glucose from the diet or from hepatic gluconeogenesis of protein and fat. Following exercise, glycogen repletion in the horse is slow compared with that in humans and the level may not be fully replenished until after 72 h (Lacombe *et al.*, 2004) or even longer depending on diet (Snow *et al.*, 1987). Glycogen repletion is dependent on the activation of glycogen synthase by substrate availability and insulin. Low plasma insulin responses can limit muscle glycogen synthesis in man (Zawadzki *et al.*, 1992), so feeding practices that promote insulin responses could be beneficial post exercise. The insulin response to forage is almost certainly highly dependent upon the amount of WSC (Borgia *et al.*, 2011, Shepherd *et al.*, 2012) and probably also the proportions of different sugars, since glucose seems to promote a higher insulin response than fructose in horses (Vervuert *et al.*, 2004a, Borer *et al.*, 2012). Insulin response can also be stimulated by the uptake of amino acids and the simultaneous ingestion of protein and carbohydrates in particular can have an additive effect on insulin response in humans compared with the ingestion of only carbohydrates (Rabinowi *et al.*, 1966, Zawadzki *et al.*, 1992, van Loon *et al.*, 2000).

In efforts to prevent the negative health effects of feeding high levels of starch, several studies have investigated exercise response after replacement of starch by fat (Hintz *et al.*, 1978, Hambleton *et al.*, 1980, Pagan *et al.*, 1987, Taylor *et al.*, 1995) or fibre (Palmgren-Karlsson *et al.*, 2002, Jansson and Lindberg, 2012, Mesquita *et al.*, 2014). After being esterified, free fatty acids can enter the muscle cell and, via β-oxidation, *i.e.* same metabolic pathway as used for VFAs (Figure 1), form acetyl-coenzyme-A for aerobic energy production yielding ATP. Reported results of fat supplementation in response to exercise include lower exercise heart rate (HR) (Pagan *et al.*, 1987), less lactate accumulation (Pagan *et al.*, 1987), higher lactate concentrations at fatigue (Taylor *et al.*, 1995), higher blood glucose concentration during exercise (Hambleton *et al.*, 1980, Taylor *et al.*, 1995), higher pre-exercise muscle glycogen (Hambleton *et al.*, 1980) and decreased glycogen utilisation (Pagan *et al.*, 1987) compared with a starch-rich diet. Since the fermentation of dietary fibre produces VFA, this is an indirect way of feeding more fat, so similar results could be expected in studies replacing starch with fibre. Mesquita *et al.* (2014), Palmgren-Karlsson *et al.* (2002) and Jansson and Lindberg (2012) all found that on replacing starch with fibre, lactate response to exercise decreased. Furthermore, Jansson and Lindberg (2012) reported lower concentrations of insulin and higher concentrations of glucose and acetate in plasma during exercise, the latter indicating more substrate available for aerobic energy metabolic pathways.

However, after replacing soluble carbohydrate with dietary fibre, lower levels of glycogen were observed by both Mesquita *et al.* (2014) and Jansson and Lindberg (2012). The latter reported 13% lower glycogen levels both pre- and post-exercise, while Mesquita *et al.* (2014) found that glycogen repletion 72 h post exercise was significantly lower in the high-fibre, low-starch diet. In contrast, post-exercise muscle glycogen was found to be higher in horses fed a diet where oats had partly been replaced with molassed sugar beet pulp and brewer's grain in the study by (Palmgren-Karlsson *et al.*, 2002). Despite these findings, the lower insulin response to feeding forage than to feeding concentrates (Connysson *et al.*, 2010) and the results reported by Jansson and Lindberg (2012) imply that decreased glycogen storage and replenishment might be a limitation to feeding a forage-only diet to athletic horses. The practical importance of a 13% decrease in muscle glycogen is still unknown. Previous results indicate that it should be no limitation to short, high intensity work (Davie *et al.*, 1996), but the effects on horses performing repeated exercise bouts with short intervals or prolonged endurance exercise still need to be investigated.

Interestingly, there are indications that the composition of the forage may affect glycogen storage in horses. In a study by Essen-Gustavsson *et al.* (2010), a forage-only diet containing 160% of CP requirements resulted in muscle glycogen levels of 630 mmol/kg dry weight at rest and 536 mmol/kg dry weight 90 min post exercise, which are similar to the levels in horses fed high amounts of concentrates (Jansson and Lindberg, 2012). This might be due to increased insulin response and indicates possibilities to adjust forage composition in order to optimise a forage-only diet for horses performing different types of exercise.

2.3 Racehorse training

2.3.1 Standardbred racing in Sweden

For Swedish Standardbred horses, training usually starts as 1-year-olds with breaking, followed by gradually increased exercise loads. About 65-70% of the 2-year-old horses pass a preparation race (2140 m, 10.5-11.5 m/s). According to Swedish regulations, the horse must have been in training for three months before the race and the last preparation races are run in October each year. Before a horse is allowed to participate in true races, it has to pass a qualification race (2140 m, >11.4 m/s for 2-year-old horses and >11.8 m/s for 3-year-old horses). The most common race distances for 3-year-old horses are 2140 m (92% of races) and 1640 m (6% of races) (Swedish Trotting Association, 2014). About 60-70% of the Standardbred horses in Sweden race at least once during their lifetime (Arnason, 1999, Swedish Trotting Association, 2014).

2.3.2 Effects of training

In order to enhance performance, training of Standardbred race horses aims to improve both locomotor and aerobic capacity. The principle of training is that by repetitive loads and slight overloads, organs may adapt by alterations of their function and composition. In Standardbred horses, both V_{200} (velocity at HR 200 beats per minute (bpm)) (Leleu *et al.*, 2005b) and V_{La4} (velocity at lactate concentration of 4 mmol/L blood) (Courouce *et al.*, 1997, Leleu *et al.*, 2005b, Lindner, 2010) are correlated with race performance. These variables indicate the importance of aerobic capacity for the type of exercise Standardbred horses perform. The maximum aerobic capacity (VO_{2max}) is dependent upon the uptake of oxygen from the lung alveoli to the blood, the transportation of oxygen to the muscles and its uptake and use by the muscle cells. Improvement of VO_{2max} by training is a widely recognised effect (Evans and Rose, 1987, Evans and Rose, 1988, Knight *et al.*, 1991) and relates to alterations within both the cardiovascular system and muscle tissue.

Muscle response to training

Muscle adaptations to training have been examined in a number of studies. Five weeks of intensive training are reported to decrease muscle fibre type IIA area by 19% and increase capillary density by 17% (Essen-Gustavsson *et al.*, 1989), both modifications that promote increased oxygen exchange between blood and muscle cells. No alterations in muscle fibre composition could be detected by those authors, but other studies have found that following sub-maximal exercise training, the proportion of muscle fibre type IIA increases at the expense of type IIB (Lindholm and Piehl, 1974, Guy and Snow, 1977, Roneus *et al.*, 1993, Tyler *et al.*, 1998).

An increase in the type IIA/IIB ratio has been reported after 16 weeks of training at 60-100% of VO_{2max} (Tyler *et al.*, 1998), after 10-15 weeks of mixed aerobic and anaerobic training (Guy and Snow, 1977) and after 8 weeks of high intensity interval training (Wilson *et al.*, 1987). The IIA fibres are fast-twitch highly oxidative fibres with high glycolytic capacity and an intermediate resistance to fatigue (Votion *et al.*, 2007, Valberg, 2014). A high IIA/IIB ratio has been associated with elite performing Standardbred race horses (Essen-Gustavsson and Lindholm, 1985). However, to achieve an adaptation in muscle fibre composition, the training intensity must be adequate (Lindholm *et al.*, 1974a). Exercise at 100% of VO_{2max} is reported to facilitate the activation of type II fibres more than exercise at 60 or 80 % of VO_{2max} in Thoroughbred horses (Yamano *et al.*, 2006). Another study found that training Arabian horses at 40% of VO_{2max} for 6 weeks did not result in any alterations in muscle composition, whereas exercise at 80% of VO_{2max} resulted in a 28% increase in oxidative type IIA-fibres (Sinha *et al.*, 1991). Moreover, Yamano *et al.* (2010) found indications that interval training tends to activate type II fibres to a higher extent than continuous exercise at the same intensity.

The muscle metabolism can also adapt to aerobic training by increased oxidative enzyme activity and increased number of mitochondria. Tyler *et al.* (1998) obtained a 29% increase in VO_{2max} by training unconditioned horses 5 days/week at 60% of VO_{2max} for 7 weeks and then at 80-100% of VO_{2max} for 22 weeks. Runtime to fatigue increased by 125% and both VO_{2max} and runtime were found to be correlated with mitochondrial volume, which increased by 130% during the training period.

In the previously mentioned study by Essen-Gustavsson *et al.* (1989), the enzyme citrate synthase increased by 27% after only one week of intensive training and by 42% after 5 weeks of training. This oxidative enzyme is an important catalyst in the tri-carboxylic cycle that produces ATP from acetyl-coenzyme-A during aerobic conditions. Increases in citrate synthase with training, thereby indicating increased aerobic capacity, have been reported in several studies (Guy and Snow, 1977, Essen-Gustavsson *et al.*, 1983, Lovell and Rose, 1991, McGowan *et al.*, 2002).

With improved aerobic metabolism, the oxidation of fatty acids may also increase at the expense of glycogen utilisation. A marker for fat oxidation is the enzyme HAD (3-hydroxyacyl-CoA dehydrogenase), which is a catalyst in the β-oxidation of fatty acids. Higher levels of HAD have been observed in Standardbred horses with good racing records than in inactive horses of the same breed (Essen-Gustavsson and Lindholm, 1985) and also in high performing endurance horses than in poorer performers (Rivero *et al.*, 1995). The evidence on the effects of training on HAD is inconsistent, but Lovell and Rose (1991) reported a 35% increase in HAD activity after 6 weeks of low and moderate training intensity. Conversely, others have found no effect of training on HAD activity (Essen-Gustavsson *et al.*, 1989, Roneus, 1993, Roneus *et al.*, 1993).

In contrast, the enzymes active in anaerobic metabolism may decrease with aerobic training. Lactate dehydrogenase, which catalyses the conversion of pyruvate to lactate, has been associated with poorer performance in endurance horses (Rivero *et al.*, 1995). A decrease in enzyme activity has been reported both after longer periods of training (32 weeks), (McGowan *et al.*, 2002) and after shorter periods (5 weeks) (Guy and Snow, 1977, Essen-Gustavsson *et al.*, 1989).

Cardiovascular response to training

The cardiovascular system may respond to training by improved oxygen transport capacity through the increase in red blood cells and blood volume (Persson, 1967, Persson and Ullberg, 1974) and also by increased cardiac output (Evans and Rose, 1988). Ventilatory capacity has only a limited ability to respond to training (Thomas *et al.*, 1983, Art and Lekeux, 1993). A significant correlation between blood volume and haemoglobin content and track performance in Standardbred horses was demonstrated by Persson (1967). Exercise haematocrit is also positively correlated to VO_{2max} (Kearns *et al.*, 2002b). Total blood volume and haemoglobin concentration may both increase with training (Persson, 1967), but haemoglobin only increases up to a certain level (Engelhardt, 1977). This is logical, since a high concentration of red blood cells can alter blood viscosity and impair circulation (McGowan and Hodgson, 2014).

The training-induced increment in cardiac output is mainly achieved by increased stroke volume (Evans and Rose, 1988) and following training, both heart size and stroke volume may increase (Engelhardt, 1977, Young *et al.*, 2005). After seven weeks of increased training loads, Evans and Rose (1988) reported a 57% increase in cardiac output and a 50% increase in stroke volume. This was accompanied by a 23% increase in VO_{2max}. Furthermore, in Thoroughbreds heart size is correlated to race performance and the relative cardiac dimensions are affected by the race discipline in which the horses are competing (Young *et al.*, 2005).

In order to achieve an improvement in VO_{2max}, submaximal training seems to be sufficient. Knight *et al.* (1991) studied the effect of training at 40% or 80% of VO_{2max} and found that both intensities resulted in a similar increase inVO_{2max} which was detectable after only two weeks. Art and Lekeux (1993) reported an increase in VO_{2max} of 25% after 9 weeks of training. The difference was significant already after 3 weeks of light exercise although the last 6 weeks with higher training intensity, causing exercise HR >200 bpm and plasma lactate concentrations of between 6-8 mmol/L, resulted in most of the increment in VO_{2max}.

Long-term effects of training

Few studies have assessed the long-term effects of training on parameters affecting aerobic capacity in Standardbred horses. Roneus *et al.* (1992) studied a group of Standardbred horses in training from 18 months of age (18-21 months: slow trot ≤5 times/week, from 21 months: addition of fast trot 1600-2000 m 2 times/week) and compared them with a group of untrained horses by muscle biopsies obtained at 3-4 years of age. The trained horses had a lower percentage of type IIB muscle fibres and higher concentrations of citrate synthase. However, there were no differences in the concentrations of HAD and lactate dehydrogenase. Four horses of each group were also sampled more frequently and in these horses the trained group showed an increase in type I fibres, a decrease in type IIB fibres and an elevated concentration of citrate synthase. In a later study, Roneus *et al.* (1993) sampled muscle biopsies from 8 horses during four years. All horses were trained from the age of 18 months (5-6 km slow trot 2-4 times/week and 1600-2000 m fast trot 2 times/week). At 50-59 months of age, muscle fibre type IIA had increased from 29 to 53 % and type IIB fibres had correspondingly decreased from 55 to 30 %. The enzyme activity of citrate synthase had increased by 24 mmol/kg/min and lactate dehydrogenase activity had decreased by 1059 mmol/kg/min. Although no correlations were found between race performance and muscle characteristics, the authors reported that the two horses with the lowest selection indices, based on performance and heritability of performance traits, also had the highest amount of type IIB fibres. In a third study (Roneus *et al.*, 1994), seven Standardbred horses in conventional training from 15 months of age (15-18 months: slow trot, 18-22 months: slow trot 5 times/week and fast trot 1000 m 1 time/week, from 22 months: fast trot 1000 m 2 times/week and slow trot on the other days) performed submaximal exercise tests at 24, 26, 29 and 40 months of age and also tests at maximal workload at 24 and 29 months of age. After 16 months of training, but not before, all horses showed a decreased lactate response and mean plasma lactate response after the submaximal test decreased from 14.5 to 7.2 mmol/L. The plasma lactate level in the maximal work load test was not correlated with speed. Between 24 and 29 months of age, muscle fibre type I increased by 4%, type IIB decreased by 7% and citrate synthase activity increased by 7 mmol/kg/min.

In a 10-month study, Persson *et al.* (1983) compared five yearlings that were trained at trot or canter five days a week with five controls that were only exercised spontaneously in a paddock. At the end of the study period, the yearlings that had been trained had increased their oxygen uptake at HR 200 and V_{La4} and had decreased their post-exercise blood lactate concentration compared with the controls.

Training-induced overload of musculoskeletal tissue

While repeated exercise-induced stress can improve metabolic and cardiovascular capacity and induce tissue adaptation, excessive exercise loads may be associated with increased risks of musculoskeletal injuries. In both Standardbred and Thoroughbred race horses, the most common health problem appears to be lameness (Vigre *et al.*, 2002, Dyson *et al.*, 2008). In a study of 275 Standardbred and Thoroughbred horses performing poorly for unknown reasons, 202 were diagnosed with musculoskeletal abnormalities (Morris and Seeherman, 1991). Even mild (grade 1-2) lameness can reduce VO_{2max} and increase lactate accumulation rate during exercise (Parente *et al.*, 2002).

A large volume of high speed training has been associated with an increased risk of musculoskeletal problems in both Standardbred and Thoroughbred race horses (Estberg *et al.*, 1995, Gaustad *et al.*, 1995, Hamlin and Hopkins, 2003) and is believed to cause accumulated clinical or subclinical bone damage (Hill *et al.*, 2001, Parkin, 2008). Young horses are more susceptible to fatigue- and microdamage, since the skeleton is immature and thereby less stiff (Stover *et al.*, 1992). In a retrospective study of Thoroughbreds in race training, two months with a cumulative increase of high intensity training predisposed them to musculoskeletal injuries (Estberg *et al.*, 1995). In Standardbred horses, the introduction of speed training is associated with a predisposition to carpal injury (Steel *et al.*, 2006).

Nevertheless, bone, cartilage and tendons may all respond to training by altered composition (Firth, 2006). During training, bone remodels to increase bone density and surface (Young *et al.*, 1991, Sherman *et al.*, 1995, Nunamaker, 2002). Cartilage may respond to training by more fibrillation, decreased stiffness (Murray *et al.*, 1999) and increased synthesis of proteoglycans (Bird *et al.*, 2000). Moreover, training of young horses can increase the thickness of the superficial digital flexor tendon (Perkins *et al.*, 2004). Even so, knowledge of training regimes that promote optimal strength and injury resistance in locomotive tissues is still lacking. To the best of my knowledge, no long-term study on the effects of different training strategies on health and performance has been conducted to date.

2.3.3 Methods to measure training response

In equine exercise physiology research, several methods are used to determine physiological response to exercise. Treadmills are frequently used for standardised exercise tests and also for conditioning. However, this section only presents the methods used in this thesis, which were chosen for their applicability in field experiments. In general, field tests have been shown to have good reproducibility in French Standardbred trotters when performed on the same track (Dubreucq *et al.*, 1995), as was done in the present study. However, differences in the physiological response to different tracks have been reported (Courouce *et al.*, 1999). This may be relevant for the change of track during winter/summer conditions in the present study. However, by testing equally many horses from each group at the same time (all horses exercised in groups), the risk of unbalanced conditions was minimised.

Lactate threshold and post-exercise blood lactate concentration

During increased workload, blood lactate increases in an exponential manner (Lindholm and Saltin, 1974, Persson and Ullberg, 1974) and high levels of lactate are associated with muscular fatigue (Engelhardt, 1977, Votion *et al.*, 2007). The lactate concentration reflects the utilisation of anaerobe metabolic pathways. Decreased lactate response to a standardised workload indicates higher adaptation to aerobic work, where more energy is derived from oxidative phosphorylation and β-oxidation, and possibly also improved lactate metabolism. At exercise intensities of HR 120-150 bpm, muscle work almost entirely utilises aerobic pathways (Lindholm *et al.*, 1974a, Persson and Ullberg, 1974, Engelhardt, 1977, Bayly *et al.*, 1983). The onset of blood lactate accumulation represents the workload at which anaerobic energy-producing pathways become more important. Onset normally occurs at a concentration of 4 mmol lactate/L blood (Persson, 1983), corresponding to approximately 55% of VO_{2max} (Evans and Rose, 1987). The velocity at a blood lactate concentration of 4 mmol/L is termed V_{La4} and has been found to be highly correlated to performance in Standardbred horses (Courouce *et al.*, 1997, Leleu *et al.*, 2005b, Lindner, 2010). V_{La4} can be calculated using exponential regression from blood lactate concentrations derived at increasing speeds (Persson, 1983, Courouce, 1999). Since maximum speed is not required during this test, it is also preferable as it lowers the risk of injuries due to excessive loads.

There are several reports regarding the effects of training on V_{La4}. In Standardbred horses, Gottlieb-Vedi *et al.* (1995) reported a 20% increase in V_{La4} in Standardbred trotters after 3 months of interval training at velocities of 7-11.5 m/s, while Persson (1983) reported a 74% increase in V_{La4} in Standardbred yearlings trained for 10 months.

Post-exercise blood lactate concentration has also been used by several authors to determine metabolic response to workload (Persson, 1983). When used repeatedly during a standardised exercise test, it may also indicate training response. Milne *et al.* (1977) trained horses for 113 days at increasing intensities and measured several variables, but the only one that significantly changed with training was post-exercise blood lactate concentration, which decreased by approximately 1.2 mmol/L. Similarly, Art and Lekeux (1993) reported a decreased plasma lactate concentration of 1.3 mmol/L after 12 weeks of increased training. Bayly *et al.* (1983) trained horses for 11 weeks and observed a decrease in lactate response to exercise during the first 36 days, but then the difference declined. As mentioned earlier, this parameter was also used by (Roneus *et al.*, 1994) to determine physiological training progress in Standardbred horses in training until 40 months of age.

Heart rate

Both HR during exercise and recovery HR are reported to be correlated (r = 0.9 and 0.5, respectively) to fastest winning times in Standardbred horses (Marsland, 1968), which makes measures of HR of great interest when assessing fitness in these horses. A decrease in working and recovery HR may be due to increased stroke volume (Ohmura *et al.*, 2013) and cardiac output (Evans and Rose, 1988), total blood volume (Persson, 1967), increased capillary density in skeletal muscles (Essen-Gustavsson *et al.*, 1989) and an increase in muscle oxidative capacity and in the ratio of type IIA/IIB muscle fibres (Roneus *et al.*, 1992). Maximum HR is strongly correlated with VO_{2max} (r=0.95) and working HR also has a strong correlation with VO_{2max} (r=0.88) (Evans and Rose, 1987).

The effect of training on working HR has been reported in numerous studies. After 11 weeks of training at varying intensity levels, working HR at a constant speed in an exercise test decreased by almost 40 bpm and the difference was significant already by day 36 (Bayly *et al.*, 1983). Similarly, Thomas *et al.* (1983) reported a decreased working HR of 10-20 bpm after 5 weeks of training. Working HR may also be expressed as velocity at HR 200 bpm (V_{200}), a parameter that can be used to determine individual training progress. However, V_{200} may not be a good parameter for comparing fitness between individuals, since individual maximum HR may show great differences and thus V_{200} will represent different work intensities (% of maximum HR) in different individuals (Hodgson, 2014).

Although well-established in man (Kenney *et al.*, 2012), exercise-induced resting HR bradycardia has been difficult to confirm in horses (Skarda *et al.*, 1976, Engelhardt, 1977, Milne *et al.*, 1977, Thomas *et al.*, 1983, Evans, 1985). Bayly *et al.* (1983) reported a numerical decrease in resting HR of 9 bpm following 11 weeks of training, but the change was not significant. However, Betros *et al.* (2013) recently reported a decrease of 6-8 bpm following 8 weeks of training. Measurement of true resting HR can be difficult, since HR <200 bpm is easily affected by excitement and stress (Persson, 1967, Engelhardt, 1977).

2.3.4 Methods to evaluate health

Clinical examinations have previously been used for systematic evaluations of health in young Swedish Warmblood horses (Jönsson *et al.*, 2013). Those examinations included palpatory orthopaedic health and locomotion, including a flexion test. Locomotion was evaluated on a straight line at walk and trot by hand, both unprovoked, and in trot after a 60-s full limb flexion test in all four legs. The health scores in the 4-year-old Warmblood horses tested affected both lifetime performance and longevity (Jönsson *et al.*, 2014).

However, subjective lameness evaluations are limited by the skill of the evaluator and may differ between observers. At low-degree lameness, the verdict may differ between veterinarians, and in fact for lameness ≤1.5 on the AAEP scale, veterinarians may only agree in 33-50% of cases (Keegan *et al.*, 2010). Since the performance of the locomotor system is crucial to the use of horses and since lameness is such a commonly occurring problem among sport horses, researchers have been working for years on developing tools that can objectively quantify the locomotive characteristics of horses. Force plates that measure ground reaction force during the stance phase of the stride and video-based motion analyses on a treadmill have both been applied (Merkens and Schamhardt, 1988, Peloso *et al.*, 1993). However, use of these systems is limited by small surface area in the case of the force plate and great requirements for resources in the case of video analyses (Keegan *et al.*, 2004). In the present study, a wireless sensor-based system (Lameness Locator, Equinosis LLC) was used for assessment of locomotion symmetry. The advantages of this system are that it permits continuous evaluation and can be used in field conditions. The system uses three inertial sensors which are attached to the dorsum of the pelvis, dorsum of the head and dorsum of the right distal forelimb. The sensors on the head and pelvis contain two single-axis accelerometers and the sensor on the forelimb contains one single-axis gyroscope. The sensors simultaneously transfer the data recorded wirelessly via Bluetooth to a computer. The differences in vertical movements of head and pelvis are presented as maximum difference and minimum difference. An early version of the system has been validated by comparison with video-based gait analyses (Keegan *et al.*, 2004) and showed high correlations in both front and hind limbs (r^2=0.95 and 0.82, respectively). The system has also been validated by comparison with force plate analysis for front limb lameness (Keegan *et al.*, 2012) and with subjective lameness evaluation by veterinarians (McCracken *et al.*, 2012). The correlation when compared with the force plate was slightly lower than when compared with video-based analysis, but still high (r^2=0.81 for vector sum of head movement). In gradually increasing lameness (induced by a screw in the sole of the hoof that was gradually tightened), the veterinarians identified the lame leg faster than the sensor system in 8% of the trials and in 33% of the trials the sensor system and the veterinarians picked out the lame leg at the same time. However, in 58% of the trials the sensor system picked out the lame leg faster than the veterinarians. All of these three studies validating the sensor-based lameness evaluation system have concluded that it seems to be a valuable tool for lameness evaluations in field conditions.

An additional method that can be used to evaluate health retrospectively among horses is the concept of days lost to training (Rossdale *et al.*, 1985, Olivier *et al.*, 1997, Dyson *et al.*, 2008, Lonnell *et al.*, 2014). With this quantification system, all health events severe enough to cancel training (depending on the trainer's judgment) are included. To identify the health problems causing days lost to training, the days lost can then be divided into different diagnosis groups (Olivier *et al.*, 1997, Dyson *et al.*, 2008). This gives an indication of the amount of medical/orthopaedic problems causing lost training days.

3 Aims of the thesis

The general aims of this thesis were to determine whether it is possible to get 1.5-year-old Standardbred horses into race condition as 3-year-olds without feeding any starch-rich concentrates but instead a forage-only diet and to evaluate the effects of a reduced high intensity training distance on performance and health.

Specific objectives were to:

- Document growth, body development, muscle glycogen content, health status and ability to reach conventional performance goals in 16 Standardbred horses fed a forage-only diet from breaking and allocated to either a control training programme or a training programme with a 30% reduction in the high intensity training distance.

- Assess the insulin response to feeding in horses fed forage with varying crude protein content, both at rest and after exercise, to get a better understanding of the impact of forage composition on insulin response that might influence performance-related parameters, such as muscle glycogen.

- Evaluate the effects of two high intensity training distances on physiological parameters such as $V_{La4,}$ blood lactate and cardiovascular response in Standardbred horses trained with the goal of racing them as 3-year-olds.

- Compare health status, locomotion symmetry and amount of days lost to training in horses subjected to either a control or a reduced high intensity training programme.

- Study whether health, locomotion qualities, body composition and physiological response to training have any associations to being fit for racing.

- Quantify the amount of training to which Swedish 2- to 3-year-old Standardbred horses are generally submitted.

The hypotheses tested in the five different studies on which this thesis is based were that:

1. It is possible to get Standardbred horses fed a starch-free, forage-only diet into condition to qualify for races and race as 3-year-olds while maintaining a body condition and resting muscle glycogen content within the normal range of athletic horses (**I** and **IV**)
2. Crude protein content of forage affects the plasma insulin response at rest, but not after exercise (**V**).
3. Horses fed a forage-only diet suffer from fewer feed-related health problems than reported in the literature (**III**).
4. Horses subjected to a reduced training programme may not qualify for races and race to a lesser extent than horses subjected to a control training programme (**IV**).
5. Reducing the high intensity training distance by 30% can result in less health problems and no differences in physiological response to exercise at the time horses are expected to race as 3-year-olds (**II** and **III**).
6. Health, locomotion qualities, body composition and physiological response to exercise affect the ability of Standardbred horses to become fit for races (**II**, **III** and **IV**).
7. The high intensity training distances to which Swedish 2- and 3-year-old horses are generally submitted are greater than the experimental training distances used in the study (**IV**).

4 Materials and Methods

This thesis is mainly based on a 2.5-year study of 1.5- to 3-year-old Standardbred horses (Study 1) fed a forage-only diet and allocated to two different training programmes. The study was conducted at the National Centre for Trotting Education, Travskolan Wången in Alsen, Sweden. It started in September 2010 and ended on 31 December 2012. Papers **I** and **IV** focus primarily on describing the growth, body development and performance in horses fed a forage-only diet. In Paper **II,** the effects of different training strategies are investigated with respect to physiological responses, while in Paper **III** health is evaluated and compared between horses trained by the two different training strategies. Paper **V** is based on a complementary study in adult horses (Study 2) conducted to get a better understanding of the impact of forage composition on insulin response, which can influence performance-related parameters such as muscle glycogen content.

4.1 Horses

In summer 2010, a letter was sent out to a group of Swedish breeders of Standardbred horses asking for stallions born in 2009 for participation in Study 1. Sixteen horses from five breeders were initially chosen based on the criteria that they were healthy and mainly of American bloodlines. Horses from the same breeder and by the same sire were preferred. During the first 3 months of the study, two horses were excluded due to health problems and replaced with horses from breeders that already had horses included in the study. The horses used from November in 2010 were from four different breeders, with eight sires, and had a mean age at the start of the study in August of 464 ± 31 days (range 400-518 days). At the end of December as 1-year-olds, all horses were castrated.

On 18 March, as 2-year-olds, the 16 horses were allocated to one of two different groups. These groups were balanced to be as equal as possible with respect to breeder, genetic parameters, exterior defects, muscle fibre composition and X-ray findings (**II**).

In Study 2, six conditioned Standardbred geldings aged 5-11 years were used (**V**).

4.2 Feeding and management

Horses in Study 1 were fed grass forage *ad libitum* (**I-IV**), complemented with pelleted lucerne and a commercial mineral and vitamin supplement depending on nutrient content in the forage. The forage was harvested in Enköping, Sweden, conserved in wrapped big bales and selected with the main emphasis on energy content. In total, seven batches were used during the study and these had a content of 10.2-11.7 MJ ME and 93-164 g CP (Table 2). Feed intake was recorded on an individual basis for 3 consecutive days (**I** and **IV**) on 15 occasions. *Ad libitum* was defined as a minimum of 2 kg of leftovers. Feed analysis was performed as described in Papers **I** and **IV** and analysis of amino acid content (Table 3) as described in Appendix 1.

Horses were kept stabled for part of the study period in individual 9 m^2 boxes for 16 h per day 4 days per week, and spent the rest of the time outside in a paddock (~20 000 m^2) with access to shelter. Water was offered inside from two 20-L buckets and outside from a large tub that was heated during wintertime. Rugs were used during wintertime (October-March) in the first and third winter, but sparingly in the second winter. Hoof trimming and shoeing was performed approximately every 5 weeks with mainly iron shoes, but also other types according to the trainer's preferences.

In Study 2, horses were fed a restricted diet calculated to correspond to 19 MJ ME/100 kg BW and day (**V**). Three different batches of haylage with similar estimated content of ME, but differing content of CP (10-15%), were fed for one week each according to a Latin square design (3 diets x 3 weeks, 2 horses on each diet in each week). Feed samples were collected on the last day of each study period.

Table 2. *Nutrient content of haylage used during the study in feed samples drilled from bales and grab samples after opening. Percentage of dry matter (DM), metabolisable energy (ME), crude protein (CP), digestible crude protein*[1] *(Dig CP), neutral detergent fibre (NDF), water-soluble carbohydrates (WSC), sugar fractions and the macrominerals calcium (Ca), phosphorus (P) and magnesium(Mg). All values are given in g/kg DM except for DM and ME*

Horse age, years	Month	DM, %	ME, MJ	CP	Dig CP	NDF	WSC	Glucose	Fructose	Sucrose	Fructans	Ca	P	Mg
1 year	August	50	11.0	152	111	545						3.6	3.2	1.2
2010	December	56	10.2	130	91	600	36	21	14	0	1	3.1	2.3	1.3
2 years	January A	62	11.0	138	98	519	56					5.6	2.4	2.2
2011	January B	59	10.3	149	109	532						5.3	2.7	2.1
	March/May	61	10.8	164	123	514	79					5.6	2.8	2.2
	June/August	62	10.7	142	102	535	41					5.5	2.7	2.3
	September/October	71	10.5	140	100	498	110					4.7	2.1	1.8
	December	67	10.4	134	94	522	84	10	42	4	27	4.4	2.3	2.1
3 years	March	68	10.9	153	112	516	83					4.7	3.0	1.7
2012	May	68	11.5	144	104	460	103					4.9	3.2	1.7
	June A	55	10.8	156	115							5.2	3.2	1.7
	June B	65	11.3	119	80	513	86	28	44	10	11	4.3	2.6	1.3
	July	60	11.7	154	113	483	96	33	54	0	0.9	4.3	2.1	1.3
	August	47	11.1	143	103	551	9	2	5	3	0	4.1	2.9	1.5
	September	66	11.2	99	62	565	164					3.6	2.3	1.1
	October	64	10.8	93	56	569	131	30	57	3	37	4.2	2.7	1.4
	December	67	11.6	114	76	521	135	31	65	2	32	4.0	2.8	1.2

[1]Calculated as (CP in g per kg DM/100) x 93.9 – (313/CP in % of DM) (Jansson *et al.*, 2011).

Table 3. *Amino acid content in forage fed* ad libitum *to 16 1.5- to 3-year-old Standardbred horses in training, g/kg dry matter*

Amino acid	1 year 2010 December	2 years 2011 December	3 years 2012 December
Cysteine	0.9	0.6	0.7
Histidine	2.5	2.8	2.7
Serine	1.3	2.0	3.1
Arginine	5.0	3.7	4.9
Glycine	5.7	5.7	5.0
Aspartic acid	9.4	10.8	8.2
Methionine	2.5	2.5	2.2
Glutamic acid	9.8	9.4	8.5
Threonine	2.8	3.5	3.9
Alanine	6.4	7.4	5.8
gamma-Aminobutyric acid	1.5	3.3	1.7
Proline	4.9	6.6	5.0
Ornithine	1.0	4.8	1.7
Lysine	4.0	5.8	5.2
Tyrosine	3.5	4.2	4.2
Valine	6.6	7.1	6.2
Isoleucine	4.6	4.8	4.0
Leucine	7.7	7.9	7.2
Phenylalanine	5.1	5.1	4.7

4.2.1 Growth and body development

To monitor growth rate in horses in study 1, height at withers and croup was recorded once a month for the first six months and then every 2-3 months (**I** and **IV**). To assess fattening and muscle size, body condition was evaluated using a 9-degree scale according to Henneke *et al.* (1983) and subcutaneous fat at two sites of the croup and also thickness of the *musculus longissimus dorsi* were measured by ultrasound on the same occasions as height measurements. The measurement of *m. longissimus dorsi* was made immediately above the 18th rib by the first lumbar vertebra (Figure 2), where a correlation between muscle area and race performance has been reported previously (Dobec *et al.*, 1994). The position was verified before the study on a pony which was euthanised for other reasons and then dissected. The mean of three measurements was calculated for each individual on each occasion.

Body weight (BW) was recorded once a week in Study 1 and Study 2 (**I**, **IV** and **V**).

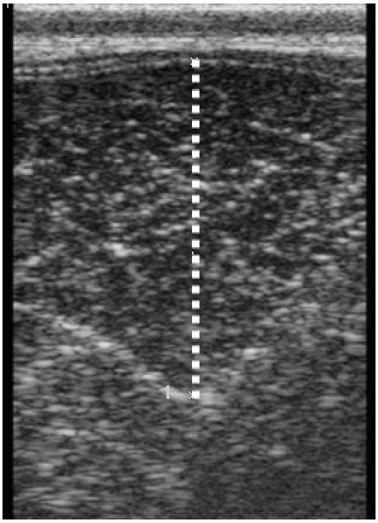

Figure 2. Measurement of *musculus longissimus dorsi* thickness by the first lumbar vertebra. Measurement is illustrated by the dotted line.

4.2.2 Insulin response to forage intake

In Study 2, blood samples were collected on the last day of each period before and at 20, 40, 60, 90 and 120 min post-feeding 15% of the daily amount of forage in the morning and 45 min post exercise. Plasma was analysed for insulin and glucose.

4.2.3 Muscle glycogen content

In Study 1, muscle biopsies were taken after ≥ 48 h of rest from the *musculus gluteus medius* according to Lindholm and Piehl (1974) in December as 1- and 2-year-olds and in November as 3-year-olds (**I** and **IV**). The samples were analysed for glycogen content according to Lowry and Passonneau (1973) after boiling in 1 mol/L HCl for 2 h.

4.3 Training and training response

The horses in Study 1 were cared for and driven mainly by high school students supervised by professional trainers (**I-IV**). The main training track used was a 1000 m banked sand track that was covered with crushed snow and fluted ice during wintertime. Training started in September as 1-year-olds with breaking (**I**) and was then gradually increased (Figure 3). In February (**II**), race track training in bouts of 1600 m was introduced 1-2 times/week. Beginning on 18 March 2011 as 2-year-olds, in horses allocated to the R-group all high intensity training sessions, defined as expected to cause a heart rate (HR) >180 beats per minute (bpm), had the training distance reduced by approximately 30% (Table 4). The HR limit of 180 bpm was chosen since lower intensities are not likely to cause a sufficient training response (Lindner *et al.*, 2009), whereas training at 178-204 bpm has been shown to result in improvements in both HR and metabolic (lactate) responses (Gottlieb-Vedi *et al.*, 1995). High intensity training was performed 1-2 times/week as race track training, interval training and, for 3-year-olds, uphill interval training (Figure 3 and Table 4), mixed with jogging 1-2 times/week. The drivers aimed for the same speed in both groups and during jogs and interval training (first 4 intervals), horses from both groups exercised together. The training programmes were designed together with a group of professional trainers. On a few occasions, as preparation for participation in official races, track training of 2000 m was performed by all horses, since most official races in Sweden are 2140 m.

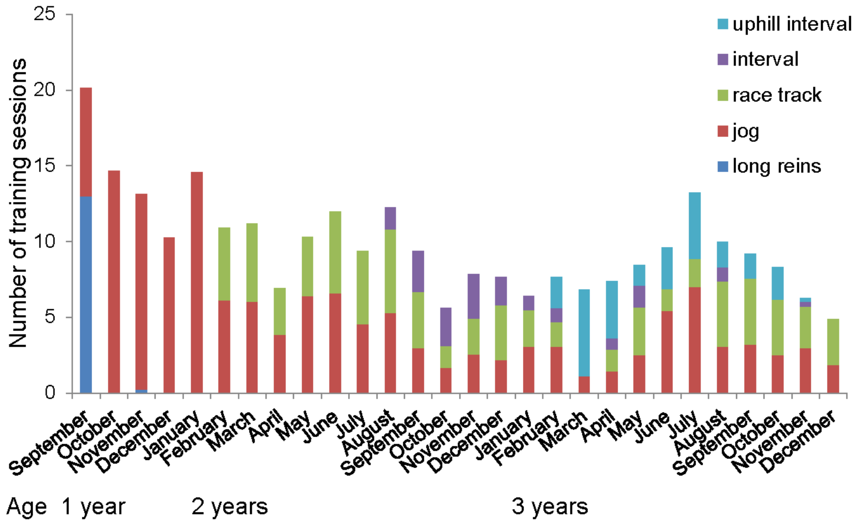

Figure 3. Number of training sessions performed and amount of different training types used for 16 Standardbred horses trained from September as 1-year-olds with the goal of racing as 3-year-olds.

Table 4. *Training conducted in 2- to 3-year-old Standardbred horses allocated to either a control (C-group) or a reduced (R-group) training programme from 18 March as 2-year-olds. GPS data for speed collected from sections of heart rate (HR) meter data with HR >180 beats per minute. Values are presented as lsmeans ± SD*

Training type	**C-group**	**R-group**
Race track training*	**1-3 x 1600 m**	**1-3 x 1100 m**
Number of training sessions/horse, 2-year-olds	41 ± 14	47 ± 7
Number of training sessions/horse, 3-year-olds	28 ± 6	28 ± 10
Speed @ HR >180, 2-year-olds	8.9 ± 0.7 m/s, n=144	8.6 ± 0.9 m/s, n=180
Speed @ HR >180, 3-year-olds	9.6 ± 1.1 m/s, n=84	9.5 ± 1.1 m/s, n=100
Warm-up	~ 3000 m, ~6 m/s	
Cool-down	~ 1200 m, ~6 m/s	
Interval training, 1 min walk in between**	**6 x 700 m**	**4 x 700 m**
Number of training sessions/horse, 2-year-olds	11 ± 3	13 ± 3
Number of training sessions/horse, 3-year-olds	6 ± 1	5 ± 1
Speed @ HR >180, 2-year-olds	8.4 ± 1.2 m/s, n=23	8.3 ± 0.8 m/s, n=26
Speed @ HR >180, 3-year-olds	9.5 ± 1.1 m/s, n=19	9.2 ± 1.0 m/s, n=24
Warm-up	~ 3000 m, ~6 m/s	
Cool-down	~ 1200 m, ~6 m/s	
Uphill interval training, 5% incline, 4 min walk/jog in between	**6 x 600 m**	**4 x 600 m**
Number of training sessions/horse, 3-year-olds	21 ± 8	28 ± 7
Speed @ HR >180, 3-year-olds	7.5 ± 0.7 m/s, n=91	7.1 ± 0.9 m/s, n=119
Warm-up	5500-6000 m, hilly track, ~5 m/s	
Cool-down	Walk 500 m downhill	

*3 x 1600 or 1100 m only performed as 3-year-olds.

**Interval in August and September as 2-year-olds was 500 m, thereafter 700 m.

Based on the correlation between post-exercise lactate response and 10-min recovery HR (y = 4.1513x + 55.944, R^2 = 0.54) observed in these horses in March 2010 (Figure 4), in all training sessions as 2-year-olds a maximum 10-min post-exercise HR of 80 bpm was aimed for (corresponding to a blood lactate concentration <6 mmol/L; plasma lactate <8 mmol/L; Stefansdottir *et al.*, 2012).

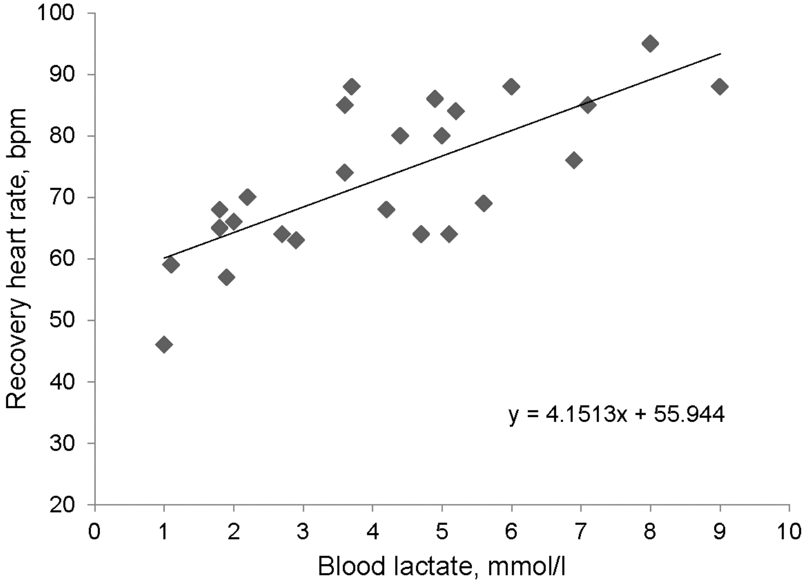

Figure 4. Correlation between recovery heart rate 10 min post exercise and post-exercise blood lactate concentration determined from blood samples collected within 1 minute post exercise in two 1600-m bouts performed at a speed of 9.3 and 9.9 m/s as 2-year-olds, r=0.74, P<0.0001.

Horses that the trainer deemed not fit were left out of training for as long as he thought was needed (**III**). According to a routine checklist that included the driver's opinion on fitness and general condition, horses were sent for a veterinary check for acute causes or if the driver scored the horse ≥2 (scale 1-3, where 1 = good, 2 = slightly asymmetric but trainable, 3 = too asymmetric to train) three times in a row. Mean number of sessions per horse and month and proportions of each training type are shown in Figure 3.

Horses in Study 2 were exercised once a week with 1600 m of high intensity training after 4 km of warm-up.

4.3.1 Performance

To reflect the Standardbred racing industry, the overall goal for horses from both groups in Study 1 was to start competing in races as 3-year-olds. When horses were considered to be adequately trained (trainer's opinion), they participated in a preparation race (2140 m, velocity standardised to 10.5-11.4 m/s) as 2-year-olds, in a qualification race (2140 m, >11.8 m/s) as 3-year-olds, and also in official races (1600-2140 m) as 3-year-olds. In official races, the experimental horses were mostly driven (78% of the races) by two professional trainers employed by the national trotting school.

For comparisons of racing results (**IV**), two reference groups were created consisting of:

1. All Swedish Standardbred horses in the cohort of 2009 (3611 horses) that were registered at a professional or amateur trainer on 1 February 2011 (2408 horses).
2. The five older siblings (same mother) closest in age to the experimental horses. These sibling horses had been trained by a number of other trainers and had been fed a conventional cereal-based diet.

In addition, earnings/start for the experimental horses in these races were compared with the driver's earnings/start during two years prior to the present study.

To study differences between experimental horses that qualified for races early and horses that qualified late, two sub-groups were formed. The six first horses to pass a qualification race (before June 21) were taken to form an early qualification group and the six last horses to pass a qualification race (after September 19) a late qualification group.

4.3.2 Standardised exercise tests

In Study 1, two types of standardised exercise tests were performed (**II**). Both tests were performed on a 1000-m race track and the horses were tested in groups of 2-7, balanced between C-group and R-group horses.

The first test was designed as a single bout of 1600 m (1600-m test), since the shortest race distance in Sweden is 1640 m and to avoid exercise at distances >1600 m. This test was performed with the aim of monitoring training response (Roneus *et al.*, 1994) with respect to lactate production, haematocrit and recovery heart rate from March as 2-year-olds. It was performed at a steady speed of 10.8 m/s five times in horses as 2-year-olds and five times as 3-year-olds. Immediately after the test, blood was sampled from the jugular vein for lactate and haematocrit determination, and recovery HR was recorded after 10 minutes (HR10).

The second exercise test was performed with the aim of determining V_{La4} of each individual horse (Persson, 1983). The test was run in four bouts of 1000 m with increasing speed, after which blood was sampled and lactate concentration and haematocrit determined. HR at V_{La4} was estimated from the last 30 s of each 1000-m bout in the V_{La4} tests and calculated using exponential regression. The V_{La4} test was performed five times in the horses as 3-year-olds.

In Study 2, a standardised exercise session was performed on an oval track with 0.6% incline on the sampling day at the end of each study period. The exercise consisted of 15 minutes of warm-up, a 1600-m bout in trot at 11 m/s and a slow trot back to the stable. Post-exercise blood lactate concentration was 12.1 ± 5.5 mmol/L (mean ± SD) and mean HR 15 min post exercise was 105 ± 6 bpm.

4.3.3 Heart rate recording

In Study 1, heart rate and track speed during all training sessions and exercise tests were measured using heart rate synchronised with a GPS (Polar CS600X and G5 GPS sensor, Polar Electro, Finland) (**I** and **II**). From HR recordings, sections where HR was >180 bpm were used to calculate total time with HR >180 bpm, total distance with HR >180 bpm and average speed when HR was >180 bpm (**II**). Recovery HR and the highest recorded HR for each horse and period were also determined from the HR curves. Curves with missing values of more than 20 s in the HR range >180 bpm were discarded. In total, before discarding there were 2003 HR recordings for the training sessions. After removal of records of poor quality, 1093 (55%) remained. In these, 874 also contained information on distance and speed and 943 had a record for HR 10 min after exercise.

Resting HR was recorded by two different methods in January in the horses as 2- and 3-year-olds and in December as 3-year-olds (**II**). HR was determined both by auscultation with a phonendoscope and by fitting horses with HR meters in boxes during night time when quiet. From the HR meter output, the lowest mean of at least 10 min of a consecutive recording was used as resting HR.

4.3.4 Cardiovascular assessments

In Study 1, measurement of blood volume were performed in December 2011 as 2-year-olds using Evans blue dye dilution as described previously (Lindinger and Ecker, 2013) and a haematocrit value obtained from the last V_{La4} sample (V_{La4} test performed on the same day) (**II**).

Left ventricular internal diameter during diastole (LVIDd) and aorta diameter were recorded using ultrasound and calculated as recommended by Patteson (1996) in January 2011 in the horses as 2-year-olds and in December 2012 as 3-year-olds (**II**).

4.3.5 Stride length

In November/December, as 1-year-olds, horses in Study 1 were equipped with the Pegasus Limb Phasing System (ETB Pegasus, United Kingdom) for gait analysis during exercise on the race track. Gyroscopic inertial movement sensors (size 72 mm x 35 mm x 18 mm, 54 g) were attached to the lateral cannon bone on all four legs. The driver was equipped with a GPS unit, which was synchronised with the sensors before data collection using ETB Poseidon software (ETB Pegasus, United Kingdom). All horses warmed up for 5 km in a slow trot (~3 min/1000 m) on a racetrack and finished with a 200-m speed where the horse was allowed to trot at fast as it could without galloping. Data from the speed were downloaded and speed, stride length and stride duration were calculated using the Poseidon software. In cases of gallop, only data from before the gallop were used.

4.3.6 Trainer survey

In August 2014, 50 Standardbred trainers were contacted to participate in a survey. The trainers were all registered as professional trainers and were chosen based on the criterion that they had trained the largest number of horses of all professional trainers in Sweden. The trainers were asked about how they performed high intensity training with 2- and 3-year-old horses in terms of training type, length of exercise bouts, number of exercise bouts/session and number of high intensity training sessions per week.

4.4 Health

To assess health of the horses in Study 1, four different methods were used (**III**);

1. All training sessions were documented continuously by the trainer and days lost to training due to health problems were chosen as an indicator of health status (Rossdale *et al.*, 1985, Olivier *et al.*, 1997, Dyson *et al.*, 2008, Lonnell *et al.*, 2014).
2. All occasions where the trainer consulted a veterinarian for examination of a horse were documented, as well as veterinary treatments and diagnoses (classified as orthopaedic injuries or medical disorders).
3. Clinical examinations were performed by a clinician blinded to the group affiliations of the horses on nine occasions using the protocol used in the 4-year-old testing of Swedish Warmblood horses described previously by Darenius *et al.* (1983) and Jönsson *et al.* (2013).

4. Objective evaluation of locomotion symmetry with a sensor-based system was performed on 17 occasions (see below).

4.4.1 Locomotion symmetry

Locomotion symmetry of each individual horse in Study 1 (**III**) was evaluated by the sensor-based system Lameness Locator (EQUINOSIS®, Columbia and St. Louis, MO; www.equinosis.com). The horses were equipped with sensors as described by McCracken *et al.* (2012) and trotted by hand for about 50 m back and forward. Analyses were performed using Lameness Locator Software (EQUINOSIS®), which presents the difference in movements of head and pelvis as maximum difference and minimum difference. From these the vector sum (VS) was calculated as VS = $\sqrt{(\text{maximum difference}^2 + \text{minimum difference}^2)}$ for both head and pelvic sensors (VS front (VSf) and VS hind (VSh), respectively).

The impact of locomotion symmetry (VSf and VSh) on physiological response to exercise was studied using the 1600-m test and the V_{La4} test. V_{La4} data from four test occasions (October excluded due to no data on VS in that month) and post-exercise blood lactate, haematocrit and HR10 data from seven test occasions (March, May, July, August and December as 2-year-olds and August and December as 3-year-olds) were used. Locomotion symmetry measurements performed within two days (except for two occasions where there were 10 and 15 days between measurements) from the test exercise tests were used for the analyses.

4.5 Calculations and statistical analyses

Statistical analyses were performed according to the descriptions in Papers **I-IV** or as stated below, using SAS 9.2 (**I**) and SAS 9.3 (**II-V**) (SAS Inst. SAS Inc. Cary, NC, USA). Differences were considered significant at P<0.05. In general, the GLM procedure was used for analyses of differences between groups or periods with one observation/individual. For analyses of parameters with repeated measurements in the same individual, the procedure MIXED was used with the covariance structure that best fitted the data depending on measurement interval. When possible and considered relevant, a start value was included in the model as a covariate. Correlations were tested using Pearson’s correlation test.

Effects of locomotion symmetry on V_{La4}, post-exercise lactate, haematocrit and recovery HR10 were tested in a mixed model including effect of occasion as a fixed effect and VS for front or hind limbs as a continuous variable.

In Study 2, intake of CP, WSC and different sugars was calculated on an individual basis for each sampling occasion. Based on individual CP intake, two CP intake groups were formed, with ≤180 g/100 kg BW and >180 g/100 kg BW (n=5 respectively). Similarly, two groups were formed based on individual intake of WSC excluding fructan (WSC-f) (≤100 g/kg BW and >100 g/kg BW). For insulin and glucose concentrations, area under curve (AUC) was calculated in GraphPad Prism (GraphPad Software, La Jolla, USA).

The GLM procedure was used when assessing the effect of CP group and WSC-f group on plasma insulin and glucose. To assess differences before and after exercise, and within and between intake groups, a Tukey's test was also used.

5 Main Results

5.1 Performance, Study 1

5.1.1 General

All 16 horses passed a preparation race between May and October as 2-year-olds and 15 horses passed a qualification race between May and November as 3-year-olds. This was more than in the cohort reference group or in the sibling reference group (Table 5) (**IV**). Nine horses participated in races between July and December as 3-year-olds, which was not different from the cohort and sibling reference groups. There was no difference in number of races between the siblings and experimental horses. The sires of the siblings had a higher breeding index than the sires of the experimental horses (111 ± 1 vs. 107 ± 1, P=0.04). When the earnings of study horses were compared with earlier earnings of the drivers, it was found that experimental horses earned more/race than earlier horses driven by the same drivers. Mean high intensity training distance for all horses to qualification was 245 ± 55 km and to first race 267 ± 42 km (mean ± SD).

Table 5. *Performance of experimental horses and horses in the two reference groups and earnings/race by the same drivers during two years prior to the study*

	Study horses	Reference cohort	Reference siblings	Horses driven by the same drivers
Preparation race	100%	77%*	84%*	-
Qualification race	94%	63%*	69%*	-
Raced before 4	56%	54%	54%	-
Number of races	1.6 ± 0.5	-	2.9 ± 0.5	-
Earnings/race, SEK	4541 ± 65	-	-	3242 ± 65*

*Indicates a significant (P<0.05) difference between experimental horses and reference groups.

5.1.2 Effect of training programme

There was no difference in the number of horses that passed a preparation race (8 horses in C-group and 8 horses in R-group), qualification race (8 in C-group and 7 in R-group) or that raced (5 in C-group and 4 in R-group). There were also equally many horses from each training group that qualified early (3 in C-group, 3 in R-group) as qualified late (3 in C-group, 3 in R-group).

5.2 Body development, muscle glycogen and insulin response

5.2.1 Feed intake and growth

During the 15 intake recording sessions, the horses consumed forage corresponding to 1.7-2.8% DM of BW (**I** and **IV**). This resulted in a daily mean ± SD energy intake of 123 ± 16 MJ ME/500 kg BW. Mean BW increased by 100 ± 6 kg (Figure 5), height at withers increased by 12 ± 1 cm, height at croup increased by 9 ± 1 cm and body length increased by 12 ± 1 cm. Thickness of *m. longissimus dorsi* (Figure 6) varied during the study between 37.0 ± 0.9 and 41.5 ± 0.9 and in December as 3-year-olds was a similar size as in September as 1-year-olds (41.4 ± 0.9 and 40.8 ± 0.9 mm, respectively). Subcutaneous fat thickness at site 1 (see Figure 6) was greatest at the start of the study, 4.4 ± 0.2 mm, but thereafter varied between 1.8 ± 0.2 and 2.6 ± 0.2 mm. Fat thickness at site 2 varied between 5.0 ± 0.5 and 8.3 ± 0.5 mm and mean ± SD for the whole study was 6.7 ± 2.1 mm. Mean body condition score (BCS) varied between 4.6 and 5.1 and individual means ranged from 4.1-5.7 (Figure 6).

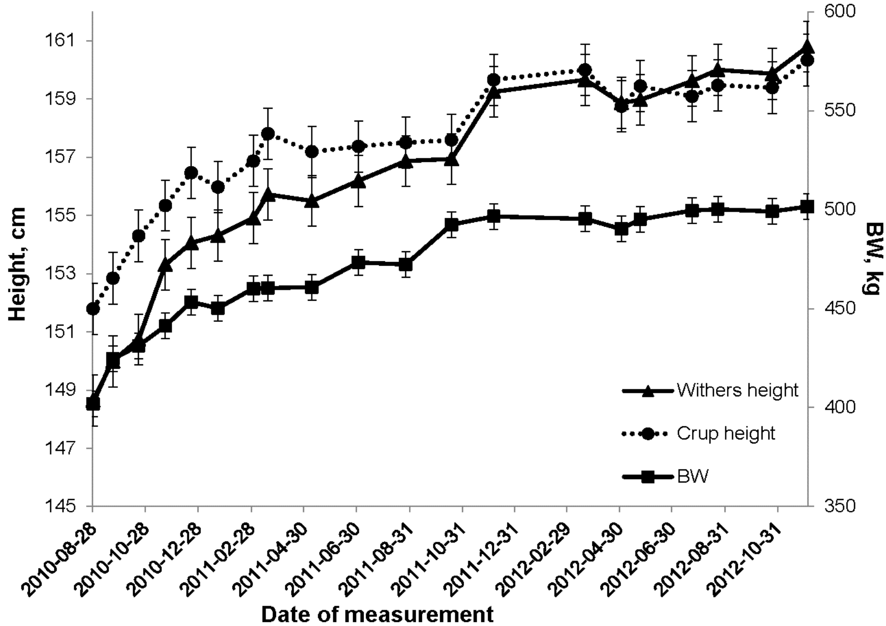

Figure 5. Height at withers, height at croup and body weight (BW) in 16 1.5- to 3-year-old Standardbred horses in training fed a forage-only diet.

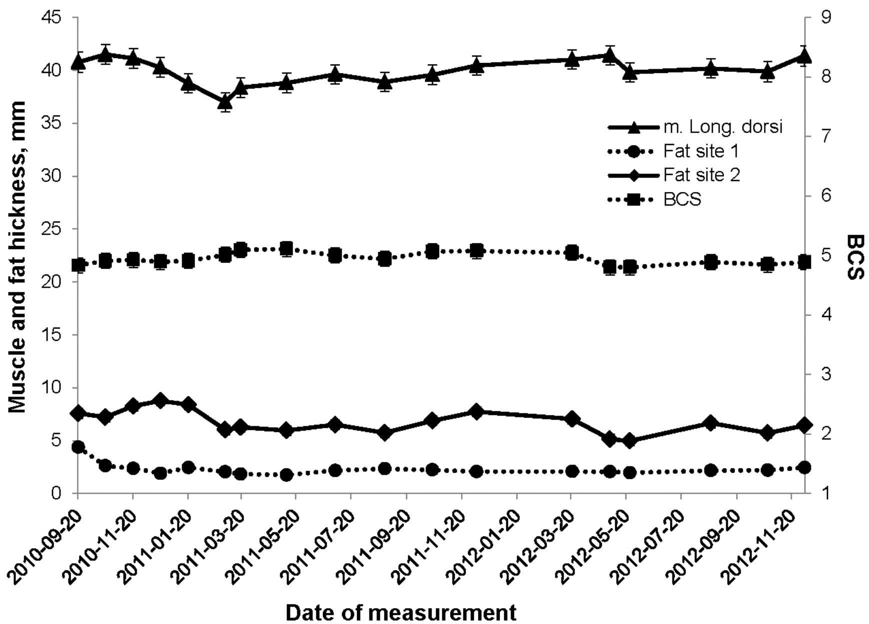

Figure 6. Thickness of *musculus longissimus dorsi* and subcutaneous fat at croup in site 1 (on top, 5 cm from midline) and site 2 (15 cm caudal of site 1) and body condition score (BCS, scale 1-9) in 16 1.5- to 3-year-old Standardbred horses in training fed a forage-only diet.

5.2.2 Muscle glycogen

There was no difference between training groups in muscle glycogen content either as 2- or 3-year-olds (**IV**). In horses rested >48 h prior to sampling, the glycogen content in *m. gluteus medius* was higher as 2- and 3-year-olds than as 1-year-olds (Table 6). As 3-year-olds, glycogen content was lower in horses rested for 48 h prior to sampling than in horses rested for >48 h (497 ± 32 mmol/kg DW vs. 620 ± 27 mmol/kg DW, P<0.01). Moreover, in the horses that exercised 48 h prior to sampling as 3-year-olds, muscle glycogen content was lower as 3-year-olds than as 2-year-olds (497 ± 32 vs. 586 ± 32 mmol/kg DW, P<0.05), but there were no difference in horses rested >48 h prior to sampling between 2- and 3-year-olds.

The glycogen content was positively correlated with BCS (r=0.34, P=0.03), BCS ribs (r= 0.37, P=0.01) and intake of fructans/100 kg BW (r=0.31, P=0.04) but no correlations were found with subcutaneous fat, muscle thickness or intake of any other nutrient component.

Table 6. *Muscle glycogen content in horses rested >48 h prior to sampling in Standardbred horses fed a forage-only diet. Different superscripts indicate a significant difference (P<0.05)*

Age	1 year	2 years	3 years
Muscle glycogen content	532 ± 17^{a}	587 ± 17^{b}	623 ± 24^{b}

5.2.3 Insulin response to forage intake

In Study 2 (**V**), the insulin AUC (area under curve) response to feed intake tended to be higher in the high CP intake group than in the low CP intake group before, but not after, exercise. There was a positive correlation between individual CP intake and insulin AUC (r=0.45, P=0.03). The ANOVA model including horse, CP group and WSC-f intake explained 95% of the variation in plasma insulin while a model including only horse and WSC-f intake explained less (87%) of the variation in plasma insulin. The creation of two groups based on WSC-f intake resulted in higher insulin AUC in the high intake group than in the low, but no differences in glucose AUC. There was no difference in glucose AUC response between CP intake groups.

5.3 Physiological response to training, Study 1

5.3.1 General

As 1-year-olds, exercise distance increased from 32 km in October to 104 km in December and the horses showed a significant decrease in recovery HR 3 min post exercise between October and December (81 and 73 bpm, respectively, P=0.03) (**I**). As 2- and 3-year-olds, the horses were exercised with HR >180 bpm for 2.69 ± 1.15 km during heat training, 3.43 ± 0.89 km during interval training and 3.11 ± 0.84 km during uphill interval training (**II**). The maximum HR during heat training was 216 ± 11 bpm, during interval training 218 ± 9 bpm and during uphill interval training 223 ± 7 bpm (mean ± SE for all horses).

Compared with the first 1600-m test in March 2011 as 2-year-olds, HR10 decreased in June 2012 as 3-year-olds, lactate decreased in October 2012 as 3-year-olds and haematocrit was higher from December 2011 as 2-year-olds (**II**). HR10 was positively correlated with blood lactate concentration (r=0.28, P<0.05) and negatively correlated with haematocrit (r= -0.41, P<0.0001).

The mean speed for all horses on reaching estimated V_{La4} was 10.7 ± 0.1 m/s when the first test was run in May 2012. V_{La4} increased in July and August but decreased in October and December compared with in May. Individual mean V_{La4} as 3-year-olds was negatively correlated with rump fat at site 1 (r= -0.58, P=0.03), BCS back (r= -0.66, P=0.01) and BCS (r= -0.66, P=0.01), but no other correlations with body measurements were found (**IV**). There was no effect of locomotion symmetry (VS front/hind) on V_{La4}, lactate, haematocrit or HR10 (P>0.05).

Resting HR decreased from January as 2-year-olds to December as both 2- and 3-year-olds when measured with phonendoscope, but only in C-group horses when measured with a HR meter (**II**). There was no effect of age on aortic diameter/100 kg BW or left ventricle internal diameter during diastole (LVIDd).

5.3.2 Effects of training programme

Lactate following the 1600-m test did not differ between training groups. There was no significant effect of training programme on V_{La4}, but there was a tendency for higher V_{La4} in R-group than in C-group horses (10.9 ± 0.1 and 10.7 ± 0.1 m/s, respectively, P<0.1).

Overall, the C-group horses showed a greater cardiovascular response to training (**II**). Following the 1600-m test, C-group horses had lower HR10 (P<0.0001) overall and in May 2011 as 2-year-olds, in August, October and December 2012 as 3-year-olds and following race track training sessions. The haematocrit levels from the 1600-m test were higher in C-group horses overall, but within occasion only in July 2011 as 2-year-olds. Haematocrit from the last blood sample in the V_{La4} test was higher (P<0.05) in C-group than in R-group horses (56 ± 0 and 55 ± 1, respectively). During training, C-group horses initially exercised for a longer time with HR >180 bpm, but after 6-9 months the difference in time declined, despite the constant difference in distance run. The speed when HR >180 bpm was higher during race track training in C-group horses than in R-group horses (9.2 ± 0.1 m/s vs. 8.9 ± 0.1 m/s, P<0.001), but there was no difference between groups during interval and uphill interval training. There was no difference in HR at V_{La4} between the groups (C-group 211 ± 3 bpm; R-group 217 ± 3). Resting HR was lower in C-group than in R-group horses, both overall and as 3-year-olds (P<0.05) when both auscultation and a HR meter were used.

There was a tendency (P<0.1) for a statistical interaction between group and age for aortic diameter, with diameter/100 kg BW increasing in C-group and decreasing in R-group horses, but there was no overall effect of group. LVIDd did not differ between groups. Blood volume in December 2011 as 2-year-olds did not differ between groups (C-group 55.9 ± 1.5 L, R-group 54.8 ± 1.5 L, P>0.05).

5.4 Health, Study 1

5.4.1 General

When managed under normal conditions, no feed-related health disorders or stereotypic behaviours were observed except for one case of oesophageal feed impaction in a horse fed dry pelleted lucerne. After this the lucerne pellets were soaked in water prior to feeding. On average, horses were examined by a veterinarian 3.9 times/year, including follow-ups after treatments (**III**). The HS in all horses decreased in May, September and December as 2-year-olds and in May as 3-year-olds compared with the scores obtained as 1-year-olds, but at the end of the study in December as 3-year-olds and in October as 2-year-olds the scores were not different from those obtained in the horses as 1-year-olds. Locomotion asymmetry in front limbs (VSf) increased in spring in horses as 2-year-olds and as 3-year-olds compared with the first measurement in September as 1-year-olds, but returned to values close to those at the start in

December as 2-year-olds and August and December as 3-year-olds. Locomotion asymmetry in hind limbs (VSh) increased from January as 2-year-olds and was higher than at the start throughout the study, with the exception of March and October as 2-year-olds and May as 3-year-olds.

5.4.2 Effect of training programme

After the split into two training groups, but not before, days lost to training were fewer in R-group than in C-group horses (17 ± 3% and 27 ± 3%, respectively, *P*=0.03) (**III**). There were no differences in number of veterinary examinations, treatments or orthopaedic diagnoses between groups. There were also no differences between groups in HS, orthopaedic remarks (OR) or lameness before (initial lameness, IL) or after a flexion test (FL). The VS values for front and hind limb did not differ between groups.

5.5 Trainer survey, Study 1

Complete answers were received from 41 trainers (**IV**). Two were excluded from the analysis because their training programme design did not include any specified high intensity training sessions. One of these used long duration exercise at slow speed and the other used a cross-country track with varying unspecified incline. The 39 trainers included in the final survey trained a total of 2401 horses, which corresponded to approximately 13% of the Swedish Standardbred horses in training. All trainers used interval training, 30 trainers used heat training and eight trainers used uphill interval training (Table 7).

Compared with the experimental training programme, the trainers in the survey appeared to use numerically more interval training and less uphill training (Table 7). Trainers used longer distances in each exercise session when performing heat training but there was no difference in interval training and uphill interval training sessions (if used). As 3-year-olds and as a mean in both 2- and 3-year-olds, planned training was less for experimental horses than stated by least half the trainers in the survey.

Table 7. *Quantification of the training programmes used in Study 1 (C-group) and by 39 Swedish Standardbred trainers, means ± SD (median)*

		Experimental horses (mean C- and R-group)			Trainer responses		
Training type		2-year-olds	3-year-olds	2+3-year-olds	2-year-olds	3-year-olds	2+3-year-olds
Heat	Part of sessions, %	79	49	64	35 ± 27	27 ± 27	31 ± 27
	Distance/bout, m	1350	1350	1350	2032 ± 382 (2000)	2032 ± 288 (2000)	2028 ± 253 (2000)
	Distance/session, m	2700	2700	2700	3284 ± 1390* (3300)	3674 ± 1254* (4000)	3489 ± 1246* (3600)
Interval	Part of sessions, %	21	9	14	58 ± 28	63 ± 29	61 ± 29
	Distance/bout, m	700	700	700	612 ± 110 (600)	638 ± 194 (600)	621 ± 134 (625)
	Distance/session, m	3500	3500	3500	3177 ± 762 (3000)	3643 ± 1250 (3500)	3388 ± 861 (3300)
Uphill interval	Part of sessions, %	0	42	22	4 ± 10	9 ± 19	6 ± 16
	Distance/bout, m		600	300	700 ± 412 (500)	655 ± 345 (525)	650 ± 134 (525)
	Distance/session, m		3000	3000	2533 ± 589 (2800)	3170 ± 595 (3500)	2910 ± 378 (2900)
	Number of sessions/week	1.8	1.8	1.8	2 ± 1	2 ± 0	2 ± 0
	Distance/week, m	5330	5403	5368	5707 ± 2553 (5600)	7434 ± 3119* (7400)	6571 ± 2320* (6700)

*Indicate a significant difference (P<0.05) in distance/session and distance/week compared with experimental training programme.

5.6 Prediction of performance, Study 1

5.6.1 Early qualification for races and race participation

Several of the health parameters affected the ability of the horses to qualify for races and race (**III**). Horses that qualified early had less asymmetric locomotion in front limbs (VS = 12.6 ± 1.0) compared with horses that qualified late (VS = 16.0 ± 1.0) (*P*=0.04). Early qualifiers were also less lame in FL (0.7 ± 0.3 vs. 1.6 ± 0.2 grades, *P*=0.04) and had a tendency for higher HS (7.6 ± 0.3 vs. 6.8 ± 0.3 points, *P*=0.06). They were also treated less frequently with joint injections (0.0 ± 0.5 vs. 2 ± 0.5 occasions, *P*=0.03) than the late qualifiers. Horses that raced went for fewer veterinary check-ups (6.3 ± 1.1 vs. 10.9 ± 1.3) and received fewer joint injections (1.2 ± 0.7 vs. 4.0 ± 0.8) than horses that did not race during the study. There was no difference in clinical examinations or VS between horses that raced/did not race.

Furthermore, the gait analysis performed as 1-year-olds on the track showed a higher speed (7.8 ± 0.3 vs. 6.6 ± 0.3 m/s, *P*=0.03) and longer stride length (4.48 ± 0.13 vs. 3.92 ± 0.13 m, *P*=0.01) in early qualifiers than in late qualifiers, but there was no difference in stride duration. Recovery HR 3 min post exercise was negatively correlated to stride length (*r*= -0.54, *P*=0.04).

There was no difference in V_{La4}, lactate response to the 1600-m test, HR10 or haematocrit post exercise between horses that qualified early or late or in horses that raced/did not race during the study period.

6 Discussion

6.1 General

The main and most clear finding presented in this thesis is that it is possible to get 3-year-old horses into race condition without feeding any starch-rich feeds from breaking (**IV**). Despite that horses in Study 1 were from sires with lower genetic potential than their siblings, and thereby could be expected to race to a lesser extent, there was no difference in part of horses that raced before four years of age. The part of experimental horses that passed preparation- and qualification races were also unexpectedly high compared with both siblings and the rest of the cohort. Moreover, a reduced amount of high intensity training proved to be just as efficient in reaching the goal of fit to race before four years of age as the control training programme. Likewise, both training programmes were equally capable of reducing lactate response to exercise, indicating improved aerobic capacity, and resulted in not significantly different V_{La4} in the horses as 3-year-olds (**II**). Although in fact, V_{La4} tended (P=0.08) to be lower for R-group horses than C-group horses. While R-group horses showed less pronounced cardiovascular adaptations to training than C-group horses, this did not affect their ability to qualify for races or race participation. When subjected to a reduced amount of high intensity training, fewer days were lost to training, indicating better health status of R-group horses (**III**). This may indicate that repeated exercise is more important for improving exercise metabolism and bringing horses to fitness for racing than training distance, at least within the limits used in the present study.

The forage-only diet seemed to promote good feed-related health (**III**). A recent study on the feeding regime of race horses in training revealed that these are being fed 4-11 kg of concentrates per day (Jansson and Harris, 2013). This increases the risk of colic by 5- to 6-fold compared with no concentrate at all (Tinker *et al.*, 1997b). Based on the prevalence of colic (8-11 cases of colic per

100 horses/year; (Tinker *et al.*, 1997a, Hillyer *et al.*, 2001)), rhabdomyolysis (6-9 cases per 100 horses/year (Isgren *et al.*, 2010, Auletta *et al.*, 2011)) and stereotypic behaviours (1-9% of horses (Redbo *et al.*, 1998)) earlier reported, it could be speculated that in the present study there should have been 3-4 cases of colic, 2-3 cases of rhabdomyolysis and one horse showing stereotypic behaviour. However, none of these problems occurred during normal management conditions, so it could be stated that horses fed a forage-only diet may suffer from fewer feed-related health problems than reported in concentrate-fed horses.

Applied together, a starch-free, forage-only diet based on high-energy forage and a reduced high intensity training distance may be beneficial to health without limiting race participation in 1.5- to 3-year-old horses.

6.2 A forage-only diet to adult and growing horses in training

6.2.1 Muscle glycogen content and insulin response

Several authors have demonstrated a decrease in muscle glycogen following high intensity exercise (Lindholm *et al.*, 1974a, Palmgren-Karlsson *et al.*, 2002, Essen-Gustavsson *et al.*, 2010, Jansson and Lindberg, 2012). A negative impact of low (70-80% depletion) levels of glycogen on performance capacity has also been demonstrated previously (Topliff *et al.*, 1983, Lacombe *et al.*, 2001). However, the effect of decreased levels of the magnitude reported by Jansson and Lindberg (2012) (-13%) in forage-only fed horses on performance capacity in the type of exercise typically performed by Standardbred race horses is not known. In this thesis, resting muscle glycogen (**I**, **IV**) was within the range reported previously in adult, trained Standardbred horses fed starch, (546 mmol/kg DW (Palmgren-Karlsson *et al.*, 2002), 572 mmol/kg DW (Jansson *et al.*, 2002) and 644 mmol/kg DW (Jansson and Lindberg, 2012)). The muscle glycogen content also seemed to be higher than reported in 2- to 3-year-old Standardbred horses (426 mmol/kg DW) by Lindholm and Piehl (1974), although 40 years of selective breeding may have changed muscle characteristics and it is unclear how those horses were fed and rested prior to sampling. The increasing muscle glycogen levels with age in horses rested for more than 48 h in the present thesis is probably due to increased level of fitness and/or age (Lindholm and Piehl, 1974).

Storage of glycogen is stimulated by insulin and thus low plasma insulin responses could limit muscle glycogen synthesis (Zawadzki *et al.*, 1992). In this thesis (Study 2), plasma insulin response to forage intake was explained to a greater extent by both WSC-f and CP content in forage than by WSC-f content alone (**V**). As indicated by the results, the post-prandial insulin response can be elevated by high CP intake in horses fed a forage-only diet, which could be expected since simultaneous ingestion of readily available carbohydrates and protein or amino acids is reported to increase insulin response (Stull and Rodiek, 1995, Urschel *et al.*, 2010). However, the intake of WSC-f explained the variation in insulin response to a greater extent than the intake of CP alone (r^2=0.87 and 0.82, respectively). In the horses in Study 1, intake of WSC and CP in November/December each year (when muscle biopsies were sampled) was numerically greater than that in the horses in Study 2, and therefore insulin response to feeding could be expected to be in the same range as in the horses in Study 1. High glycogen levels (630 mmol/kg DW) in horses fed a forage-only diet have previously been reported by Essen-Gustavsson *et al.* (2010) in adult horses fed a forage with 16.6% CP, resulting in CP intake of 319 ± 31 g/100 kg BW. This is similar to the intake in horses in Study 1 in December as 2-year-olds, but numerically lower than the CP intake in December as 3-year-olds, at which time instead intake of WSC was numerically higher. It could therefore be suspected that the relatively high intake of CP and WSC in the horses in Study 1 enhanced glycogen storage.

The conclusion reached in this thesis is that a forage-only diet appears to be no limitation to achieving high resting muscle glycogen levels. However, since post-exercise glycogen replenishment may take 72 h or even more (Snow *et al.*, 1987, Lacombe *et al.*, 2004), regular high intensity training could pose a problem. Horses in this thesis exercised 48 h prior to biopsy sampling had a lower muscle glycogen content than horses rested >48 h prior to sampling. The time of replenishment of muscle glycogen in horses fed a forage-only diet and the optimal forage composition, particularly with regard to WSC, CP and amino acid content, need further investigation.

6.2.2 Body composition

The forage-only diet resulted in moderate body condition, where BCS was maintained at 4.8-5.1 from September as 1-year-olds (**I**, **IV**). This is in agreement with earlier reports on Standardbreds in training (Gallagher *et al.*, 1992), event horses (Burk and Williams, 2008) and endurance horses (Lawrence *et al.*, 1992) and indicates that energy intake was sufficient to match energy output. Although the optimal body condition for race performance is not yet known, the importance of proper body condition for performance is most certainly not negligible. In human athletes, it has been well documented that a lean body mass is beneficial to sports performance (Maughan and Leiper, 1983, Landers *et al.*, 2000, Legaz *et al.*, 2005). In horses, a lean body mass is associated with elite performance (Lawrence, 1990, Kearns *et al.*, 2002c, Fonseca *et al.*, 2013). This is probably explained by the extra weight that fat mass contributes, thereby increasing the amount of work needed to move the body (Kearns *et al.*, 2002a). It is therefore not surprising that BCS and subcutaneous fat thickness were negatively correlated with V_{La4} in this thesis, despite the small number of horses studied. This was also found in earlier studies by Leleu and Cotrel (2006) and Kearns *et al.* (2002b). However, a BCS ≤4 is reported to affect the glycogen stores negatively (Jones *et al.*, 1992), which was confirmed by the negative correlation between BCS and muscle glycogen in this thesis, and a BCS <3 can impair performance, at least in endurance horses (Garlinghouse and Burrill, 1999).

Although the thickness of the *m. longissimus dorsi* has been reported to increase with training (D'Angelis *et al.*, 2007), its thickness was not different between training groups in this thesis and showed no correlation to V_{La4}. However, area of the *m. longissimus dorsi* has been related to lifetime earnings in a previous study (Dobec *et al.*, 1994) why it was included in the present work. Muscle thickness in locomotor muscles (*vastus lateralus/intermedius* and *extensor carpi radialis*) measured by Kearns *et al.* (2002c) did not show a significant correlation to running performance, as also found in this thesis.

6.2.3 Growth and feed-related health

Energy intake during the 15 recording occasions in Study 1 (19-31 MJ ME/100 kg BW) (**I**, **IV**) is comparable to that in horses performing heavy to very heavy exercise according to NRC (2007) (defined as race training with a mean heart rate over the entire exercise of 110-115 beats per minute). As yearlings, estimated daily energy intake in the horses in this thesis was 48% higher than the requirements suggested by NRC (2007) for 18-month-old horses subjected to moderate exercise. Since no quantifiable fattening seemed to occur, probably due to the amount of training to which the horses were submitted, perhaps in combination with the great amount of outdoor management, this may indicate that NRC (2007) underestimates the needs of yearlings in training for racing.

The horses studied here grew at a similar rate or faster than those of light breeds used in previous studies (Bigot *et al.*, 1987, Trillaud-Geyl *et al.*, 1992, Thompson, 1995). Average daily gain declined from 432 g as 1.5-year-olds to 120 g as 2-year-olds and 27 g as 3-year-olds, and the horses appeared to reach their mature BW in spring as 3-year-olds. This is earlier than what could be estimated from a report by Bigot *et al.* (1987) in horses of light breeds. While body weight gain declined in January as 3-year-olds, height at withers still increased in December as 3-year-olds, which implies that mature height was not reached before 4 years of age. As 1-year-olds, the increase in BW (12.7%) and the increase in withers height (3.6%) was numerically larger than previously described in horses of other light breeds at the same age (BW: +9.4-10.6% (Thompson, 1995), height: +1.7-3.5% (Hintz *et al.*, 1979, Trillaud-Geyl *et al.*, 1992, Thompson, 1995)) (Table 8). Scientific studies of growth rates in horses of light breeds older than two years appear to be scarce, but one comparison can be made of the increase in height at withers from August as 1-year-olds until March as 3-year-olds, which has been reported to increase by 8.5 cm (5.4%) in Danish Warmbloods at a high feeding level (Staun *et al.*, 1989). In the present thesis, the increment at the same age was 10 cm (7.4%). Although these comparisons are only numerical, they imply that growth rate was enhanced rather than limited by the feeding regime used in the present study.

An interesting observation is that as 1.5-year-olds, horses were on average 2.8 cm taller at croup than at withers (Figure 5). This is in line with the value recorded by Sandgren *et al.* (1993) (2.1 cm difference) in 14.5-month-old colts. In agreement with findings by Magnusson (1983), the difference was still obvious as 2-year-olds, but declined so that horses were about 0.5 cm higher at withers from spring as 3-year-olds.

Table 8. *Comparison of height at withers in 12- to 19-month-old Standardbreds (STB), Danish Warmbloods and Thoroughbreds (THB) in the present thesis and in other studies*

Reference	Age, months	12	14.5	15.2	18	19.2	Gain 15.2-19.2
Present thesis	Swedish STB ♂	-	-	148.7	153.3	154.1	3.6 %
Sandgren *et al.* (1993)	Swedish STB ♂	143.2	149	-	-	-	-
Staun *et al.* (1989)	Danish Warmblood	-	-	155.5	-	161	3.5 %
Valette *et al.* (2008)	French STB ♀♂	146	-	-	152		-
Valette *et al.* (2008)	THB ♀♂	146	-	-	151		-
Hintz *et al.* (1979)	THB ♂	147.2	150.2	151.8	154.5	155.4	2.4 %
Thompson (1995)	THB ♂	146.3	149.6	149.6	151.5	152.2	1.7 %
Jelan *et al.* (1996)	THB ♀♂	146	149.5	-	153	154.5[2]	3.6 %[1]

[1]14.5-20 months.

One explanation for the high growth rate in the present thesis might be that the horses had *ad libitum* access to feed. Cymbaluk *et al.* (1989) reported that horses aged 6 to 24 months fed *ad libitum* gained on average 24% more BW and 11% more height than yearlings fed according to recommendations by NRC (1978). However, in spring as 3-year-olds, when all 16 horses shared two big-bale feeding stations, we suspect that the *ad libitum* concept was inadequate. Having few feeding stations makes it crowded and some low-ranking individuals may not have had enough time to eat as much as they wanted. This could partly explain the negative energy balance observed as decreased body condition (BCS -0.2 points, rump fat 2-30% and *m. longissimus dorsi* thickness -4%) and BW (-2%) and highlights the importance of overall management to the function of this type of diet in growing horses performing heavy exercise. Furthermore, in early March 2012 we observed decreased DM intake and suspected that the hygienic quality of the forage might have deteriorated, since there was occasionally a smell of butyric acid from the forage during this period. A sample of the forage was therefore analysed for bacteria, mould and yeasts but this revealed no deviations. Other causes might have been eruption of a second molar tooth and a high prevalence of parasites in horses which also can contribute to a negative energy balance. In fact, during spring as 3-year-olds, the number of eggs per g faeces was >700 in as many as 11/16 horses. This can be rated as rich occurrence according to (Swedish National Veterinary Institute, 2014).

Occurrence of intestinal parasites can also increase the risk of colic (Reinemeyer and Nielsen, 2009), but horses in this thesis were dewormed twice a year, which probably was preventive. The case of colic that did occur during the study was related to unusual management practices, as prior to a gastroscopy the horse had been fasted for 12 h and was then sedated and examined. A couple of hours after the examination, the horse showed symptoms of colic, which were treated with evacuation of gastric air through a nasal tube and administration of intravenous analgetics after what the symptoms declined.

One case of oesophageal feed impaction occurred during the first week when the horses were fed dry pelleted lucerne. After this, the lucerne was soaked in water prior to feeding and no other cases of impaction occurred.

In summary, resting muscle glycogen content, growth rate and BCS in the experimental horses were within the range reported previously. This indicates that a forage-only diet with a high energy and nutrient content fed *ad libitum* to young Standardbred horses in training appears to be no limitation to muscle energy storage, growth and body development. Based on the results presented in this thesis, dietary inclusion of up to 100% forage with an energy content of >10.5 MJ ME/kg DM can be recommended for young horses in training for racing.

6.3 Reduced training distance in young horses

This is the first time that the physiological responses of different training programmes have been documented in Standardbred horses from breaking until expected to race at the age of 3 years (**II**). Reducing the amount of high intensity training by 30% of the distance did not affect race participation or V_{La4} and horses in R-group lost fewer days to training due to health problems than horses in C-group. However, already within the first 2-8 months of training, C-group horses displayed more pronounced cardiovascular training responses, both during training and in exercise tests. Despite the longer training distance in C-group horses, within 6-9 months of training the time spent at HR >180 bpm did not differ from that of R-group horses. In heat training, the C-group horses also trotted at a higher speed than the R-group horses at HR >180 bpm, indicating greater aerobic capacity. Following the 1600-m test and also after training, when the C-group horses exercised over a longer distance, recovery HR was still lower or equivalent to that in the R-group horses. Part of the decrease in HR might have been due to the increased haematocrit, which is reported to be positively correlated with VO_{2max} (Kearns *et al.*, 2002b). A 10% increment in haematocrit has been associated with a 5% higher circulating O_2 volume (Lykkeboe *et al.* (1977). Another part of the decrease in HR might have been due to the fact that aortic diameter tended to be larger in C-group compared with R-group horses, indicating increased central circulatory conductance. Reports of aortic adaptations to training in horses are scarce, but larger aortic dimension has been observed in human endurance athletes compared with strength training athletes (Kinoshita *et al.*, 2000).

Although HR during exercise and recovery HR have both been correlated (r= 0.9 and 0.5, respectively) with fastest winning times in Standardbred horses (Marsland, 1968), V_{La4} shows a stronger correlation with race performance than V_{200} (Lindner, 2010). This may explain why the lower circulatory capacity in R-group horses did not affect their ability to qualify for races or the number of horses that raced. Significant cardiovascular adaptations to training were observed in R-group and C-group horses. Following the 1600-m test, both groups improved (reduced) recovery HR by 17-19 beats (~20%) and haematocrit concentration increased by 17%. The importance of lower cardiovascular capacity of the magnitude observed in R-group horses compared with C-group for true run performance was not studied and remains to be investigated.

The decrease in resting HR observed in C-horses was small (-3 bpm) and when measurements were made with a HR meter no decrease in R-group horses was observed. The resting HR therefore does not seem to be a good indicator of improved cardiovascular capacity in horses, although a previous study found a greater decline following training (6-8 bpm; (Betros *et al.*, 2013). If resting HR is to be recorded, it should be noted that in the present thesis measurement with a phonendoscope resulted in a higher resting HR, probably due to excitement, than measurement with a HR meter during night-time. It can therefore be recommended to measure resting HR with a HR meter rather than a phonendoscope.

The mean distance of high intensity training in the programmes designed for the experimental horses resulted in less planned high intensity training than for at least half the horses in the trainer survey (**IV**) and shorter distances during heat training sessions. Taken together with the ability to achieve a similar V_{La4} with both C- and R-training programmes, this implies that the volume (duration) of training sessions may not be a crucial point in improving performance capacity once volume exceeds a hitherto undetermined threshold. Once above the volume threshold, intensity (speed or load; *i.e.* total muscle fibre recruitment) or repetitions (number of training sessions; (von Wittke *et al.*, 1994) are likely to be of greater importance. As the experimental horses passed preparation and qualification races to a higher extent than horses of the same cohort and older siblings and there was no significant difference in the amount of horses that raced, this indicates that the distance of high intensity training to which most young Swedish Standardbred horses are subjected could be reduced without affecting race participation negatively.

The different training programmes did not affect feed intake, body condition, muscle thickness or growth rate (**IV**). Moreover, they did not affect the amount of veterinary treatments, locomotion symmetry or clinical health status, at least not when assessed on 11 and 6 occasions over 20 months, respectively (**III**). However, C-group horses lost more days to training than R-group horses and the difference tended to be significant even when only training-related causes were included, despite the small number of observations. This indicates that the training programme to which C-group horses were submitted resulted in a greater negative impact on health than the training programme to which R-group horses were submitted. We believe that it is possible that by reducing the amount of high intensity training sessions for horses in C-group, trainers were able to keep them as healthy as horses in R-group. Trainer skill is a known factor of importance for injury prevention in horses (Vigre *et al.*, 2002, Dyson *et al.*, 2008, Reed *et al.*, 2013). However, it is also possible that the measurements of locomotion symmetry and clinical examinations were performed at too long intervals to detect all health problems causing days lost to training.

The fact that R-group horses lost fewer days to training due to health problems is probably the main reason why there was no difference in distance of high intensity training to qualification for races or to first race participation between groups (**IV**). Furthermore, there was no difference in time until fit to race, as equally many horses in R-group and C-group qualified early or late in the season as 3-year-olds.

6.4 Long-term study of training effects – health and performance

In horses in both groups, physiological alterations in several of the measured parameters could be related to training. In Figure 7, an attempt is made to illustrate possible impacts of type and amount of training on alterations in lactate response to exercise and locomotion asymmetry.

During the first five months after breaking, horses in both groups were jogged at the same and increasing distances (**I**). Already in December as 1-year-olds, an improvement in recovery HR 3 min post exercise (jog) was observed compared with in October, and exercise HR was correlated to trained distance/month. A fast response of recovery HR has been reported after only 5 weeks of training (Bayly *et al.*, 1983, Thomas *et al.*, 1983), so this could be expected. However, in horses losing days to training due to health problems, the decrease in recovery HR was less. Due to this and to the lack of correlation between HR and body measurements, training and not growth is believed to be the main reason for the decreased HR. It has been reported for growing horses that training can increase oxygen uptake at a given HR (Persson *et al.*, 1983). The training programme used in the present thesis until December as 1-year-olds was sufficient to improve fitness to a level where all horses managed to trot 5-7 km at a speed of ~5.6 m/s in a vigorous way. This training did not seem to affect health negatively, since HS in clinical examinations were high, comparable to HS reported in 4-year-old Warmblood riding horses (Wallin *et al.*, 2001, Jönsson *et al.*, 2013). However, this is likely based on pre-selected material, as the most ill horses may not show up for evaluation (Jönsson *et al.*, 2013). Moreover, locomotion symmetry, especially in the front limbs, was maintained at a steady level (Figure 7) (**I**, **III**). It could be deduced from the VS curve for hind limbs that locomotion asymmetry was increasing already from November as 1-year-olds, although not significant until in January as 2-year-olds. However, in January as 2-year-olds the horses had recently been castrated and this could probably explain some part of the increment in hind limb asymmetry at that time.

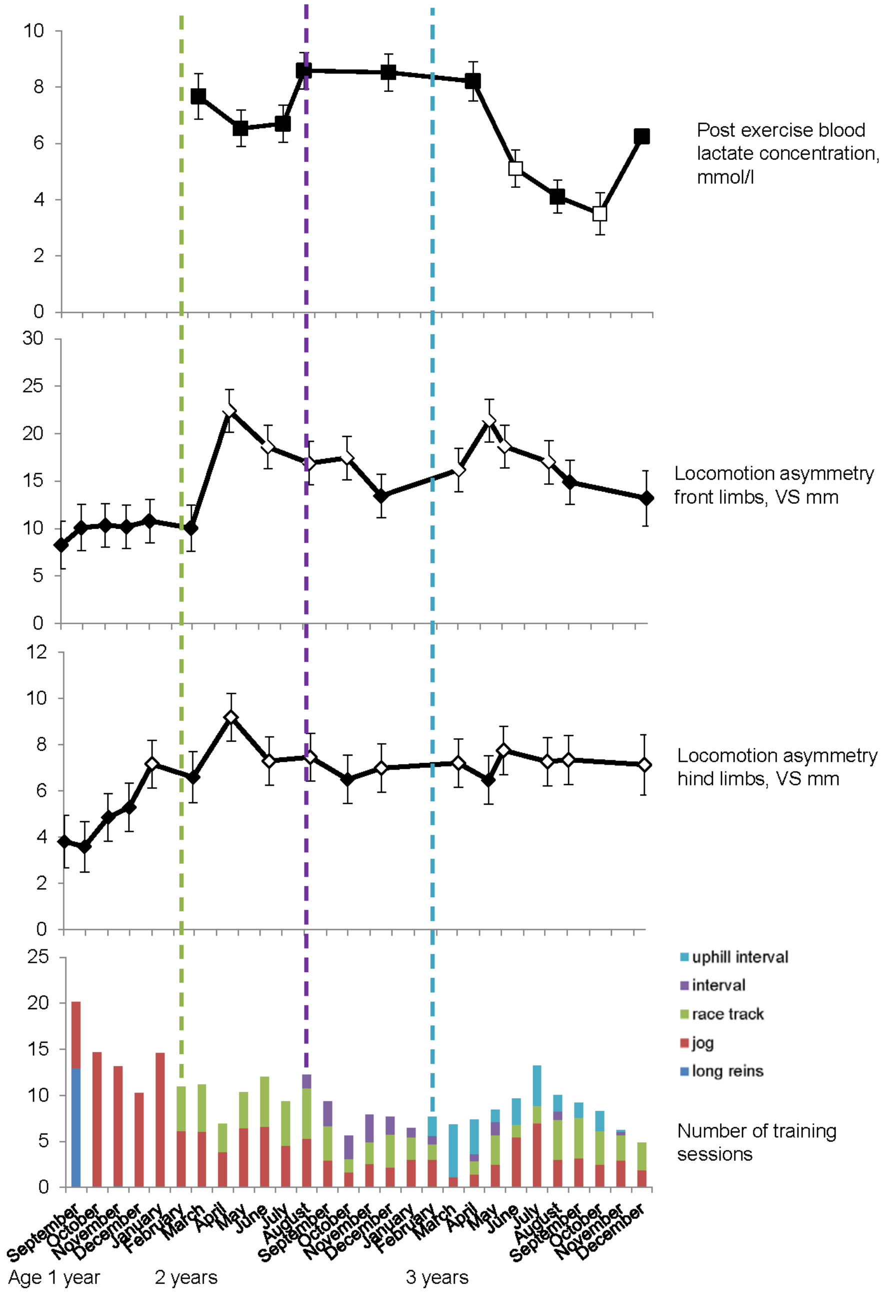

Figure 7. Synthesis of type and amount of training, locomotion asymmetry and post-exercise lactate response in the horses from breaking until December as 3-year-olds.

In February as 2-year-olds, heat training was introduced as single bouts of 1600 m at ~9 m/s (**II**). The first 1600-m test was carried out in March and after that double heats were most commonly used, at a speed of up to 11 m/s depending on track conditions. In May as 2-year-olds, quite a drastic increase in locomotion asymmetry was observed, especially in front limbs (Figure 7) (**III**). Introduction of speed training has been indicated to create a predisposition to carpal lameness in young Standardbred horses (Steel *et al.*, 2006), so the recently initiated and rapidly increasing amount of heat training is likely to be the cause. In 2-year-old Thoroughbreds, the introduction of speed training is also associated with an increased risk of musculoskeletal injuries (Cogger *et al.*, 2006). The recommendation to trainers in this study to adjust speed during heat training sessions to achieve HR10 <80 bpm was an attempt to avoid overload of tissues. Nonetheless, the increased locomotion asymmetry implies that a maximum HR10 of 80 bpm was still too high to avoid increased asymmetries connected to high intensity training and suggests that slower speeds or shorter distances resulting in an even lower recovery HR are required. After the peak in locomotion asymmetry in May, the horses seemed to recover, showing decreasing locomotion asymmetries, indicating training adaptation in the locomotive tissues.

The heat training did not result in any significant decrease in post-exercise lactate level during the following five months. However, the introduction of speed training has been reported to reduce the amount of type IIB muscle fibres in favour of type I and IIA fibres, which would imply greater aerobic capacity (Roneus *et al.*, 1992). Nevertheless, in assessing short-term training effects, measurement of lactate in response to a 1600-m test appears to be of limited value, since the concentration in the horses studied here did not decrease significantly until October 2012 as 3-year-olds, similar as reported previously by Roneus *et al.* (1994).

In contrast to the introduction of heat training, the interval training at the racetrack, which was introduced in August as 2-year-olds, did not result in increments in locomotion asymmetry (Figure 7). Instead, both VSf and VSh continued to decrease. That interval training seems to be more lenient to skeleton of young horses then heat training at longer distances has been suggested earlier by Lindholm and Saltin (1974). The interval training did not seem to affect lactate response to exercise, but on the other hand very few interval training sessions were performed (<4 sessions/month), so the effect is difficult to evaluate. Gottlieb-Vedi *et al.* (1995) found that 12 weeks of interval training in deconditioned horses 3 times/week at 2-5 x 2 min at 7-11.5 m/s, and resulting in similar lactate response as the interval training in the present thesis, resulted in > 2 mmol/L lower post-exercise blood lactate concentration and a decrease in exercise HR of 9 bpm at a constant speed of 8 m/s.

As 3-year-olds, alterations were apparent in recovery HR, lactate response and locomotion symmetry (Figure 7). It can thus be suggested that the uphill interval training, which caused the highest maximum HR of all training types, perhaps in combination with the 2000-m tests at near race speed (performed up to 10 times between January and December), greatly contributed to the decline in post-exercise blood lactate concentration and recovery HR and the increase in V_{La4} (**II**). Uphill training might be comparable to draught-loaded training, which activates type II muscle fibres (Gottlieb *et al.*, 1989) and reduces lactate accumulation (Gottlieb *et al.*, 1991).

However, the results in this thesis suggest that the heavy workload in uphill interval training also increased, although reversibly, locomotion asymmetry in front limbs and perhaps inhibited a decline in VSh (hind) as 3-year-olds (**III**). The latter is supported by the observation that VSh tended to decrease in R-group, but not C-group horses, during the last 7 months as 3-year-olds compared with the peak in May as 2-year-olds. It could be speculated that the R-group horses were about to recover, whereas in C-group horses training frequency and/or workload was too heavy for recovery. Furthermore, in March and May as 3-year-olds, two horses went lame and were later diagnosed with carpal arthritis that was severe enough to take them out of training. Even though one of these horses was also diagnosed post mortem with arthrosis, most likely caused by an injury present before the start of the study, lameness was not visible until after initiation of uphill interval training. Since exercise at an incline results in an additional force load on the hind limbs (Dutto *et al.*, 2004), it could be speculated that the seriousness of the arthritis in these horses was to some part caused by the uphill intervals. Some speculative conclusions about uphill interval training are therefore that it seems to induce great training progress in aerobic capacity, but also increases the loads on locomotion tissues, which might require more recovery compared with other training methods. In some individuals with weaknesses in limb construction, it could even cause severe damage.

The amount of uphill training and the total number of training sessions decreased during August-December and it is possible that this, at least partly, explains the increased post-exercise lactate and HR10 and decreased V_{La4} in December, as well as the decreased locomotion asymmetry in front limbs. However, other contributing factors may be poorer training conditions during winter (altered track surface, heavier shoes on horses and clothing of drivers increasing weight and air friction, for example), and possibly impaired health status (suspected infection with increased body temperatures in some horses).

Interestingly, loss of training days three weeks prior to exercise tests did not affect metabolic and cardiovascular responses significantly, since there were no effects of training status (quantity of training three weeks prior to the exercise tests) on the HR and lactate responses (**II**). Earlier findings on the effect of reduced activity on oxygen uptake, HR and lactate responses to exercise are contradictory (Thornton *et al.*, 1983, Butler *et al.*, 1991, Art and Lekeux, 1993, Gottlieb-Vedi *et al.*, 1995, Mukai *et al.*, 2006). The variation in responses between studies might be due to the level of fitness in the horses, the volume of reduction and the low number of observations in those studies.

From an animal welfare perspective, the magnitude of training-induced alterations in locomotion symmetry may be questioned in relation to enhancement of performance. However, to reach conventional performance goals, training-induced stress on cardiovascular, metabolic and musculoskeletal functions is necessary. The training programmes used in the present thesis resulted in 55% lower blood lactate concentration post exercise, 20% lower recovery HR and a high proportion (94%) of horses qualified to race. The alterations in front limb locomotion asymmetry seemed to be reversible, so it could be assumed that training-induced load was within the limits of tissue ability to cope. However, it is not known whether the lasting asymmetry observed in hind limbs was pain-induced, which would indicate a constant overload and inability of tissues to cope and thus an animal welfare problem, or whether it was the result of neurological adaptations to speed training, changing locomotion pattern permanently. Since horses with more symmetric locomotion qualified earlier for races, less alteration in locomotion pattern seems to benefit performance.

The results of this thesis also highlight the importance of gradual introduction of high intensity training forms. Since the trainer in the present study had no access to locomotion symmetry data, training could not be adjusted according to alterations in locomotion that were not detected by the trainer himself. However, it could be speculated that with knowledge about the increments occurring in spring both as 2- and 3-year-olds, training could have been adjusted to prevent further rise. In the future, studies on adjustment of training load from both metabolic/circulatory response and locomotion pattern perspectives should therefore be conducted.

6.5 Parameters affecting early performance

The division of horses into early and late qualifiers was performed retrospectively, to determine whether any of the measured parameters affected the earliness of a horse. Swedish Standardbred horses are bred for early competition (Swedish Trotting Association, 2014) and it has become more common to compete with 2-year-old horses. However, the numbers of subjects included in groups in this thesis was small (six horses in each group), so the results should be regarded with caution.

There were equally many horses from R-group as from C-group that qualified early and qualified late, so training programme appeared to not have an effect on early qualification (**IV**). Physiological variables such as post-exercise blood lactate concentration, recovery HR, haematocrit and mean V_{La4} also had no effect. All body measurements were tested for differences between late and early qualifiers, but no differences were found. However, height at withers has been correlated positively to earnings per start in Standardbred horses in a study of 500 horses (Magnusson and Thafvelin, 1990). Previous studies have also reported an effect of body composition on performance (Lawrence *et al.*, 1992, Kearns *et al.*, 2002c, Fonseca *et al.*, 2013) and VO_{2max} (Kearns *et al.*, 2002b).

Factors that affected early qualification for races in the present thesis were connected to the health and function of the locomotive apparatus. Horses that qualified early had less orthopaedic problems, verified as more symmetric locomotion in both clinical examinations and as measured by the Lameness Locator (**III**). They also required fewer joint injections during the training period than did late qualifiers. Interestingly, maximum speed and stride length as measured already as 1-year-olds affected the ability of the horses to qualify early for races as 3-year-olds. Horses that qualified early as 3-year-olds were as 1-year-olds able to increase stride length, and thereby speed, to a higher extent before breaking into a gallop or being restrained by the driver to prevent galloping than horses that qualified for races later in the season as 3-year-olds. This is an interesting observation and, together with the more symmetric locomotion of early qualifiers, may indicate that part of what is mentioned as a horse's talent may in fact be measurable as locomotion qualities and speed ability already in 1-year-old horses. It has been shown in French Standardbreds that locomotion characteristics differ between elite performers and medium performers (Leleu *et al.*, 2005a). In that study, the elite performers were at a given submaximal speed characterised by longer stance duration, higher stride frequency and longer propulsion duration. Those authors also found a positive correlation with earnings and negative correlations with record to stride length and stride symmetry. However, the findings presented in this thesis needs to be confirmed in a much larger study population.

In this thesis, the stride length (4.16 ± 0.41 m) at a mean speed of 7.18 ± 0.91 m/s was positively correlated with speed. From the correlation with speed (y = 0.4126x + 1.1973), stride length at a speed of 8.46 m/s was calculated to 4.67 m. This is very close to what Leleu and Cotrel (2006) reported (4.68 m) for 2-year-old horses at the same speed. In the present thesis, stride length was negatively correlated with recovery HR 3 min post exercise, which could imply that horses with short strides were more affected by the exercise. Correlations between gait variables and physiological responses have also been reported by Leleu *et al.* (2004), but those authors did not find any correlation for stride length and V_{200} or V_{La4}, but instead for symmetry. In contrast, in the present thesis V_{La4}, post-exercise blood lactate concentration and recovery HR were not affected by locomotion symmetry. These results are also contradictory to Parente *et al.* (2002), who found that in Thoroughbreds even mild lameness could reduce VO_{2max} and increase lactate accumulation rate during exercise.

Although physiological parameters are known to affect performance, in this small study material with a rather small variation in many parameters, locomotion qualities appeared to be more important for early performance than physiological variables. However, the goal tested in these horses, to qualify for races and race before the age of four years, is not quite the same as actual race performance (*i.e.* record, earnings, placing *etc*.). For that, it is almost certain that physiological parameters such as V_{La4} and body composition will still have a great influence (Kearns *et al.*, 2002c, Leleu *et al.*, 2005b, Leleu and Cotrel, 2006, Lindner, 2010).

6.6 Challenges and weaknesses of the methodologies

The study of young Standardbred horses presented in this thesis is unique in more than one way. This is the first time that the effects of feeding and controlled training programmes have been documented in as many as 16 horses all the way from breaking until the end of the season as 3-year-olds. This is also the first time that experimental diets and training programmes have been tested for effects on conventional performance goals such as passing preparation races, qualification races and ability to race. A study of this kind provides a great opportunity to get a better understanding of how the way in which horses are trained and fed affects them from a holistic and long-term perspective, more specifically in terms of their health and performance capacity. However, there is a reason why studies of this type are rare; they demand a great deal of resources. To make it possible to conduct the study presented in this thesis, it had to be performed with a somewhat unconventional design.

The most obvious weakness of the study design is the lack of a control group consisting of horses of the same age, trained in the same programme but fed a conventional diet with a high inclusion of concentrates. This would have required another 16 horses with all the associated resources, *i.e.* one more stable with 16 boxes, one more paddock, twice the amount of feed, twice the amount of staff, twice the amount of experimental material *etc*. The decision to perform the study without this concentrate-control was made after formulating the hypothesis that "It is possible to get 3-year-old horses into a condition to race without feeding them any starch-rich feeds". Since both practical experience and earlier reports (Jansson and Harris, 2013) show that concentrates are used by the majority of racehorse trainers, the amount of horses that make it to the race course when fed concentrates could be roughly estimated and used for comparisons.

Secondly, the work described in this thesis was performed at the National Centre for Trotting Education, which provides good facilities for Standardbred race horse training, and the experimental horses were used in the education of high school students. Unlike in a professional racing stable, the experimental horses were cared for, broken and driven by mostly young and relatively unexperienced people who could be assumed to have limited knowledge of young horses. In addition, to fit the teaching plans for the students, the horses were cared for by several different students and with separate staff during summer and winter breaks. Although the students were supervised by trainers, this implementation could be assumed to have affected the training and management of the horses and the ability to discover early stage lameness or other weak signs of health problems. However, since staffing is an enormous cost in a project like this, this solution made the project possible at all. Both the trainers and the students did a fantastic job by getting as many as 94% of the horses able to qualify for races as 3-year-olds.

Study 2 highlighted the variation that could be expected within forage batches, which is important for experimental purposes and also when feeding commercial horses that for different reasons require a very specific ration of nutrients and prevention of long-term nutrient deficiency. In Study 1, several samples were taken within the same batch and samples collected in the same 3-day-period were mixed before analysis to avoid large variations dependent upon a single sample.

7 Conclusions

The general conclusion of this study is that it is possible to get Standardbred horses fed a starch-free, forage-only diet from breaking, and submitted to a reduced training distance, into condition to race as 3-year-olds. Other conclusions are that:

- Horses fed a forage-only diet and trained at shorter distances than most horses in conventional training passed national preparation and qualification races to a higher extent than their older siblings and trained horses of the same cohort. They also started to race before four years of age to the same extent as both older siblings and the rest of the cohort.

- Horses submitted to a 30% reduced amount of training reached conventional performance goals such as preparation races, qualify for races and race to the same extent as horses submitted to a control training programme.

- By reducing the high intensity training distance, fewer days were lost to training, indicating better health status.

- A reduced training programme may result in a less pronounced cardiovascular response than a control training programme, but does not affect lactate response to exercise or V_{La4}.

- A forage-only diet may result in a maintained moderate body condition, a high growth rate and resting muscle glycogen content within the range reported and fewer feed-related health disorders than reported previously in concentrate-fed horses.

- The CP content of forage together with the content of WSC may explain more of the variation in insulin response to feeding than the WSC content alone. High contents of CP in forage may therefore promote higher insulin responses to feeding than low contents of CP.

- The introduction of new training may cause alterations in locomotion symmetry that may be reversible.

- Locomotion symmetry and stride length may affect the ability of a horse to qualify for races early.

- In this thesis it was easier to identify early qualifiers based on locomotion qualities than body composition, V_{La4}, HR, haematocrit and lactate response to exercise.

Based on the results of this study, inclusion of up to 100% of forage with a content of >10.5 MJ metabolisable energy per kg dry matter is recommended for young horses in training for races. Further, the results imply that the training distance to which most 2- to 3-year-old horses are submitted could be reduced without affecting race participation negatively and could lower the amount of days lost to training. Objective evaluation of locomotion pattern is recommended in young horses during the training phase, as this may be a predictor of future performance. Training causing long-term increases in locomotion asymmetries should be avoided from both a performance and animal welfare perspective if induced by pain.

8 Future research

This is the first time that the long-term effects of different training programmes have been documented from a holistic perspective in young race horses fed a forage-only diet. Since the horse is affected by both its genotype and multiple environmental factors, future studies should try to adopt a broader perspective when assessing the effects of diet, training and management.

The study described in this thesis concentrated on the development and performance of horses ≤ 3 years of age, but the function of a forage-only diet to adult horses during intensive periods of racing and for maximum performance still needs to be investigated. In a study resembling that described here, the forage-only diet should be compared with a traditional diet with concentrates within the same individuals with respect to race results, health, muscle glycogen content and replenishment and body condition.

Additional studies testing the forage-only concept in horses performing other types of demanding exercise, such as Thoroughbred race horses, endurance horses and eventing horses, would also be highly relevant. Since the work performed by these horses differs greatly, so do their combustion rate and energy requirements. Ways to optimise the forage, particularly with respect to energy and CP content, to suit horses of different disciplines could then be appraised together with feed scientists and the feed industry.

To get the most out of the forage-only concept, it should also be tested during the most challenging periods of breeding; pregnancy, lactation and early growth. Studies where horses are bred all the way from foetus stage to fit for competition on a forage-only diet compared with horses where both the mare and the foals have been fed concentrates would be most interesting with respect to general health, cartilage development, insulin sensitivity, feed conversion and later performance.

The results presented in thesis indicate that the amount of high intensity training could be reduced by up to 30% without affecting the ability of horses to become fit for racing. However, to get a better understanding of how modulation of training concepts affects health and performance, more long-term studies investigating factors contributing to training load, such as frequency, are required.

Studies should also be conducted on how training intensity and frequency could be modulated from information about cardiovascular and metabolic adaptation combined with information about alterations in locomotion symmetry. However, before that, more detailed studies are needed on how locomotion symmetry is affected by different types and phases of training. Studies with a greater number of horses are needed to investigate whether training alters locomotion pattern permanently and whether these alterations are connected to performance and injury rate. Further and more profound studies of how Standardbred horses are generally trained are also warranted in order to formulate practical recommendations.

Finally, it would be most interesting to continue the objective characterisation of locomotion qualities in young horses, which may be related to later performance. With knowledge about the early qualities of a horse, realistic expectations on performance could be shaped and training could be adjusted accordingly.

9 Populärvetenskaplig sammanfattning

Sverige är en av de världsledande nationerna inom travsport och varje år föds drygt 3000 varmblodiga travhästar här. Ett av avelsmålen för de varmblodiga travhästarna är att de ska utvecklas och kunna prestera tidigt i livet. Som 2-åringar är det ca 70% av varje årskull som deltar i och klarar ett så kallat premielopp vilket innebär att minst 70% av de tvååriga hästarna är i träning. Trots det så är det historiskt sett bara ca 30-50% av de treåriga hästarna som lyckas komma till start. Det är känt sedan tidigare att vanliga orsaker till att träningen inte kan bedrivas som planerat är hälsoproblem och det vanligaste hälsoproblemet hos både trav- och galopphästar är hälta. Det är också vanligt med hälsoproblem relaterade till utfodringen, som till exempel kolik och beteendestörningar, hos alla typer av sporthästar.

Den här studien har visat att ettåriga travhästar som utfodras med bara energirikt grovfoder och tränas över kortare distanser än normalt kan gå premielopp och komma till start i minst lika stor omfattning som hästar i konventionell träning utfodrade med kraftfoder samt ha en mycket god utfodringsrelaterad hälsa.

Bakgrund

Eftersom att unga travhästar både växer och tränar hårt är deras behov av energi högt. För att tillgodose detta är det mycket vanligt att man utfodrar dem med stora mängder stärkelserika kraftfoder. Kraftfoder ökar dock riskerna för flera allvarliga hälsoproblem så som kolik, korsförlamning, magsår och beteendestörningar. Genom evolutionen är hästen anpassad till att effektivt bryta ner och utnyttja energin som finns i gräs då den i grovtarmen kan omvandla fibrer till kortkedjiga fettsyror.

Muskeln kan under arbete utnyttja både fett och glukos för sin energiförsörjning. Fett förbränns under närvaro av syre (aerobt) och bildar vatten och koldioxid som slutprodukter. Glukos kan förbrännas både aerobt, då det bildar samma slutprodukter som när fett förbränns, och anaerobt, det vill säga utan syre då mindre energi frigörs och slutprodukten blir laktat (mjölksyra). Förmågan att utnyttja aerob energiförbränning kan förbättras med träning och är också kopplad till förbättrad prestation hos travhästar. Tidigare studier har visat att både utfodring med rent fett (olja) och med enbart grovfoder kan minska bildningen av mjölksyra under arbete jämfört med när stärkelse utfodras vilket indikerar att den aeroba förbränningen ökar på bekostnad av den anaeroba. Hur en foderstat helt utan kraftfoder fungerar under en längre tidsperiod till växande hästar som tränas med avseende på hälsa och prestation har aldrig tidigare studerats. Ett potentiellt problem med en foderstat med hög andel fett/fibrer är dock att den kan ge ett lägre innehåll av muskelglykogen (energilager i muskeln), kanske till följd av minskat insulinsvar efter utfodring då insulin är en viktig aktör i inlagringen av glykogen. Låga muskelglykogennivåer är en faktor som under arbete ökar risken för trötthet och minskad prestationsförmåga.

Träningen av travhästar syftar huvudsakligen till att förbättra prestationsförmåga och förmågan att motstå skador som kan uppkomma till följd av överbelastning. Trots att träningen har en så stor betydelse för hur prestationspotential kan tillvaratas och hälsan bibehållas, finns det till idag, inga långsiktiga studier där effekterna av olika träningsprogram från inkörning till startmässigt skick har undersökts.

Studier

I ett 2,5 år långt försök har 16 varmblodiga travhästar studerats ur ett holistiskt perspektiv för att öka kunskapen om hur träningsmängd och utfodring kan påverka hälsa och prestation. Studien startade när hästarna var 1,5 år gamla och avslutades i december det år de fyllde tre. Under hela studien har hästarna utfodrats med enbart energirikt grovfoder (tidigt skördat hösilage), lusern samt mineraler och vitaminer. Mätningar av hästarnas tillväxt, hull, muskel-och fettansättning, rörelsesymmetri och hälsa genomfördes kontinuerligt.

Hästarna, som sköttes och tränades av elever på travskolan Wången under handledning av tränare, kördes in i september som ettåringar. I mars när hästarna fyllde två år delades de in i två grupper som var balanserade för faktorer som skulle kunna påverka prestationen så som härstamning, muskelfibersammansättning, mankhöjd och exteriöra avvikelser. Den ena gruppen lottades sedan till ett träningsprogram där mängden högintensiv träning, så kallade snabbjobb, reducerades med 30 % av distansen. Hästarna i de båda grupperna tränades sedan på samma dagar och i samma hastigheter men olika lång sträcka och alla snabbjobb dokumenterades med pulsklocka.

Som tvååringar användes mestadels heatträning och intervallträning och som treåringar användes även intervallträning i backe. Träningen justerades så att återhämtningspulsen 10 minuter efter avslutat arbete som tvååringar inte skulle överstiga 80 slag per minut i ett försök att undvika skador till följd av överbelastning. Arbetstester för mätning av mjölksyra, andel röda blodkroppar i blodet och hjärtfrekvens genomfördes kontinuerligt. Målet för samtliga hästar var att de som tvååringar skulle genomföra ett godkänt premielopp och som treåringar bli godkända i kvallopp och komma till start.

Inom ramen för denna doktorsavhandling genomfördes också en telefonundersökning med 41 proffstränare om hur de tränar sina 2- och 3-åriga hästar då detta inte tidigare är vetenskapligt dokumenterat samt en studie på vuxna hästar i syfte att bättre förstå hur innehållet av näringsämnen, och då främst protein, kan påverka insulinsvaret efter utfodring och därmed kanske inlagringen av muskelglykogen vilket är viktigt för prestationsförmågan.

Resultat

Alla 16 hästarna klarade ett premielopp som tvååringar, 15 st kvalade som treåringar och 9 st kom till start före fyra års ålder. Både andelen av projekthästarna som klarade premielopp och som kvalade var större än hos resten av hästarna i samma årskull som var i träning samt större än andelen av projekthästarnas äldre syskon.

Under hela studien förekom inga fall av vare sig kolik, korsförlamning eller beteendestörningar så länge hästarna sköttes enligt normala rutiner. Enligt tidigare rapporter om kraftfoderutfodrade hästar skulle dessa problem kunna ha förekommit 1-4 gånger var under studien. Hullet varierade mellan 4,8-5,0 (skala 1-9) vilket stämmer bra överrens med tidigare rapporter på trav-fälttävlans- och distansrittshästar. Dock förekom individuella variationer och både höga hullpoäng och tjockare underhudsfett påverkade hastigheten när hästarna nådde sin mjölksyratröskel (när laktat börjar ackumuleras) negativt. Hästarna konsumerade hösilage i en mängd av upp till 2,8 % torrsubstans av kroppsvikten men det var viktigt att de hela tiden hade verkligt fri tillgång till foder, något som i studien definierades som att de alltid skulle ha minst 2 kg kvar i boxen. I genomsnitt resulterade foderkonsumtionen i ett energiintag av 123 MJ omsättbar energi per 500 kg kroppsvikt vilket motsvarar rekommenderat energiintag för hästar som utför den här typen av arbete. Under studieperioden växte hästarna lika bra eller något snabbare än observationer i tidigare rapporter och de nådde sin vuxenvikt under våren som treåringar.

Hästarna som tränades i det reducerade programmet uppnådde prestationsmålen i samma omfattning som hästarna i kontrollgruppen och det var ingen skillnad i hästarnas mjölksyrasvar under arbete. Dessutom förlorade hästarna i gruppen med den reducerade träningsdistansen färre träningspass på grund av hälsoproblem, något som antyder att kontroll-träningsprogrammet hade större negativ effekt på hälsan än det reducerade. Dock gav inte det reducerade träningsprogrammet samma effekt på cirkulationen som kontrollprogrammet då hästarna i den reducerade träningsgruppen hade högre puls både under och efter arbete samt högre vilopuls som treåringar. Detta skulle till viss del kunna förklaras med en något mindre andel röda blodkroppar som tvååringar och en något mindre diameter på aortan som treåringar. Trots skillnaden mellan grupperna så verkar detta inte ha påverkat förmågan att kvala eller komma till start.

Under studien undersöktes hästarnas rörelsesymmetri objektivt med sensorer då ett asymmetriskt rörelsemönster kan indikera smärta och är det vi i dagligt tal kallar för hälta. Vid undersökningstillfällena fanns det ingen skillnad i graden av asymmetri mellan de två träningsgrupperna men för samtliga hästar i studien ökade graden av asymmetri både under våren som tvååringar och under våren som treåringar. Orsaken till detta var troligtvis introduktionen av snabbjobb under våren som tvååringar och introduktionen av backträning och ökade snabbjobb som treåringar. Att anpassa träningsintensiteten hos tvååriga hästar till att resultera i en återhämningspuls på max 80 slag per minut verkar därför inte vara tillräckligt för att undvika förändringar i rörelsemönstret till följd av överbelastning. Asymmetrin för frambenen minskade dock under hösten och vintern under båda åren vilket tyder på att vävnaderna klarade av att återhämta och anpassa sig till den nya belastningen. Backträningen och de ökade snabbjobben som treåringar resulterade i märkbart lägre mjölksyrasvar under arbete. Backintervaller verkar dock kräva mer återhämtning än träning på plana banor för att inte påverka rörelsemönstret och benhälsan negativt. De hästar som rörde sig mer symmetriskt och vid färre tillfällen fick veterinär behandling för ledproblem var också de som lyckades kvala tidigt under året som treåringar. Man bör därför, både ur ett djurvälfärds- och prestationsperspektiv, undvika träning som i hög grad försämrar rörelsesymmetrin. För att i god tid upptäcka låggradig hälta kan ett sensorbaserat system liknande det som användes i studien vara till hjälp.

Tränarna som deltog i telefonundersökningen körde i genomsnitt två snabbjobb per vecka och använde sig till största delen (61%) av intervallträning. Den totala planerade mediandistansen av högintensiv träning var 6700 m per vecka vilket kan jämföras med den i genomsnitt för båda träningsgrupperna i studien planerade distansen 5368 m per vecka. Det var också vanligast att man i heatträningen använde sig av 2000 m heat, en sträcka som hästarna i studien bara gick undantagsvis då den längsta sträckan annars var 1600 m. Sammantaget så pekar projekthästarnas höga premielopps- och kvalloppsprestationer, de likvärdiga resultaten i kontrollgruppen och den reducerade träningsgruppen och de färre tappade träningsdagarna på grund av hälsoproblem i den reducerade träningsgruppen mot att man i konventionell träning troligtvis skulle kunna reducera träningsdistansen för 2- och 3-åriga hästar utan att det negativt påverkar prestationen.

Sammanfattningsvis kan vi baserat resultaten i den här studien rekommendera att man utfodrar unga hästar i träning med upp till 100 % av ett grovfoder innehållande minst 10,5 MJ omsättningsbar energi och 63 g smältbart råprotein per kg torrsubstans. Grovfodret bör vara av god hygieniskt kvalitet, utfodras med fri tillgång och kompletteras med mineraler och vitaminer. Vi kan också rekommendera att man använder sig av kortare distanser än 2000 m vid heatträning av 2- och 3-åriga hästar och troligtvis även kortare total sträcka högintensiv träning än vad som används till att träna många 2- och 3-åriga hästar idag samt att man tar hjälp av ett objektivt mätsystem för att tidigt kunna upptäcka låggradiga hältor och justera träningen därefter.

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10.1 Appendix 1

Method description amino acid analysis

Amino acid content in the feed was analysed using Waters UPLC Amino Acid Solution for feeds with AccQ•Tag™ derivatisation. In brief, 50-mg feed samples were weighed into a 100-mL Teflon liner for a Paar microwave oven. The samples were hydrolysed in 15 mL 6M HCl containing 1% phenol in the microwave oven (Synthos 3000, Anton Paar Nordic, AB Sweden) using a temperature programme set to increase to 150 °C during 5 min, hold this temperature for 30 min and cool for 15 min. For analysis of methionine and cysteine, 50-mg feed samples were oxidised with performic acid (Alternative 1: By adding to 2 mL cold, freshly prepared formic acid:perhydrol (9:1) and incubating overnight at +4 ^{o}C. Thereafter, 2 mL freshly prepared sodium bisulphite (0.17g sodium bisulphite/mL water) solution were added to each tube and the contents mixed for 15 min. The samples were then hydrolysed as described above. Alternative 2: By adding to 2 mL cold, freshly prepared formic acid:perhydrol (9:1) and incubating in a water bath at 50 °C for 1 h. Evaporate performic acid in a heating block under N_2 at 55°C to dryness.) The oxidised samples were then hydrolysed as described above.

Reference feed material was regularly analysed as an internal quality control. Within-laboratory relative variation was less than 10% for all amino acid analyses. The results were not corrected for losses during hydrolysis, which were normally low.