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Faecal K^+/Na^+ ratio in lame horses and horses with a symmetric motion pattern at trot - a potential biomarker for osteoarthritis?

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Abstract

Osteoarthritis (OA) is the most common reason for lameness in horses and is related to chronic pain. The disease is both costly for the horse owner and a welfare issue for the horse and a common cause for horses being culled. It would be helpful both for horse owners and veterinarians to find an early non-invasive biomarker to identify horses with OA. If so, it would be possible to start treatment before severe damage to articular cartilage has occurred. OA is associated with a low-grade inflammation and often affects several joints in the body. The inflammation in the joint influence several biochemical parameters, for example the chondrocytes of the articular cartilage inhibit their synthesis of extracellular matrix protein, downregulates the Na^+/K^+ pump and glucose transport to the cell.

The ***hypothesis*** of this study is that an impaired Na^+/K^+ pump activity in the inflamed cartilage of horses with OA will result in a change in K^+/Na^+ ratio in faeces. A high ratio could predict OA in horses.

The ***objective*** of this project was to collect faecal samples from symmetric and lame horses and to analyse the K^+/Na^+ ratio, to determine whether there is a correlation between the K^+/Na^+ ratio and lameness in horses associated with OA. The outcome of the study will define K^+/Na^+ ratio in faeces as a biomarker for OA or not.

In this age-matched study nine symmetric and 27 lame horses were included. Lameness Locator® was used for evaluating locomotion symmetry in healthy horses and 62 horses were measured twice (with four to six weeks in-between). In conjunction with the second measurement a faecal sample was taken. Nine horses were considered symmetric and included in the study. Lame horses were included after a clinical assessment and lameness examination including flexion test and reduced lameness after intra-articular anaesthesia. A faecal sample was taken after the examination. All horse owners responded orally to questions about the horse`s age, training status and supplementary feeding of salt.

The concentration of potassium and sodium in the faecal samples were analysed and a ratio for K^+/Na^+ was calculated from the given values. The mean age of the healthy horses included were 10,2 years and for lame horses 11,1 years. The mean (±SD) K^+/Na^+ ratio in faeces for the symmetric horses were 8,4 (±8,8) and for the lame horses 8,9 (±24,4). The mean K^+/Na^+ ratio in faeces for horses (n=10) that received salt supplement daily in the feed was 4,7 (±26,5) and the horses (n=23) that not received any supplementation was 13,2 (±5,0).

In conclusion these results show that faecal K^+/Na^+ ratio is not a suitable biomarker for identifying lameness associated with OA in horses.

Keywords: horse, osteoarthritis, K^+/Na^+ ratio, faeces, biomarker

Sammanfattning

Osteoartrit (OA) är den vanligaste ledsjukdomen hos häst som resulterar i hälta och kronisk smärta. Sjukdomen är kostsam för hästägaren, ett välfärdsproblem för hästen och är en vanlig orsak till nedsatt prestation och avlivning. En biomarkör för att identifiera hästar tidigt i sjukdomen, skulle kunna möjliggöra behandling innan kroniska broskskador utvecklats. OA är associerat med en låggradig inflammation och drabbar ofta flera leder i kroppen. Ledinflammationen påverkar flera biokemiska parametrar hos broskcellen, vilket till exempel leder till minskad syntes av extracellulära matrix proteiner, nedreglering av Na^+/K^+-pumpen och förändrad glukostransport.

Hypotesen är att en nedreglerad aktivitet hos Na^+/K^+-pumpen i inflammerade broskceller hos hästar med OA, ger en förändrad K^+/ Na^+-kvot. En hög kvot i träcken skulle kunna fungera som en biomarkör för OA hos häst.

Målet med denna studie var att samla in träckprover från friska respektive halta hästar för analys av K^+/ Na^+-kvot, för att studera ett eventuellt samband mellan hög kvot och hälta. Resultatet av studien kommer att definiera om K^+/ Na^+-kvot i träck är en lämplig biomarkör för OA eller ej.

I studien inkluderades nio symmetriska och 27 halta hästar. Medelåldern i grupperna var jämförbara. Hästar med symmetriska rörelser i trav valdes ut efter sensorbaserad mätning av rörelsesymmetri med Lameness Locator®, 62 hästar undersöktes två gånger med fyra till sex veckors mellanrum. I samband med den sista mätningen togs ett träckprov. Nio hästar bedömdes som symmetriska och inkluderades i studien. Halta hästar rekryterades genom klinisk bedömning och hältutredning inkluderande böjprov och nedsatt hälta efter intraartikulär bedövning. Ett träckprov togs vid veterinärbesöket. Hästägarna besvarade muntligen frågor om hästens ålder, träningsstatus och eventuell tillskottsfodring med salt.

Natrium- och kaliumkoncentrationen i träckproverna analyserades och en K^+/Na^+-kvot räknades ut. Medelåldern för de symmetriska hästarna var 10,2 år och för de halta hästarna 11,1 år. Medelvärdet (±standardavvikelsen) för K^+/Na^+-kvoten för de symmetriska hästarna var 8,4 (±8,8) och för de halta hästarna 8,9 (±24,4). Hästar som dagligen tillskottsfodrades med salt (n=10) hade i medelvärde 4,7 (±26,5) och hästar som inte tillskottsfodrades med salt (n=23) hade 13,2 (±5,0) i medelvärde.

Resultaten i denna studie visar att fekal K^+/Na^+-kvot inte är en lämplig biomarkör för identifiering av hälta associerad med OA hos häst.

Nyckelord: häst, osteoartrit, K^+/Na^+ kvot, träck, biomarkör

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1 Introduction

Lameness is a common condition in Swedish sport horses and the most common reason for a horse being culled (Egenvall *et al*., 2006). Osteoarthritis (OA) is the most common cause for joint pain and a reason for early retirement from training and racing (Perkins *et al*., 2002). OA starts as an inflammation in any of the joints' structures (van Weeren & Brama, 2001; van Weeren *et al*., 2016) and is a major welfare concern in the horse because of the chronic pain (van Weeren & de Grauw, 2010).

OA is not life-threatening, but this chronic progressive disease is associated with severe pain, which is the main symptom of the disease (Skiöldebrand *et al.*, 2018; Rotter Sopasakis *et al.*, 2018). The tissue inflammation induces release of pain mediators and alters biochemical parameters such as glucose uptake, glutamate release and K^+/Na^+ exchange of the chondrocytes in the articular cartilage (Skiöldebrand *et al.*, 2018; Rotter Sopasakis *et al.*, 2018). OA downregulates the Na^+/K^+ pump (Skiöldebrand *et al.*, 2018), which is in charge for the intracellular ion concentrations (Sjaastad *et al.*, 2003; Milner *et al.*, 2012; Wilkins *et al.*, 2000). In the case of inflammation, the sodium and potassium exchange of chondrocytes will be changed, resulting in an electrolyte imbalance and influx of water to the cytosol and thereby disrupt the intracellular calcium ion balance (Skiöldebrand *et al.*, 2018). The Ca^{2+} homeostasis in the cell is therefore changed (Skiöldebrand *et al.*, 2018). Therefore, analysis of K^+/Na^+ ratio may be a valuable biomarker for early stages of OA. A faecal sample may be a simple method to measure K^+/Na^+ ratio in horses (Jansson, 1999; Jansson *et al*., 2010). If there is a link between an altered Na^+/K^+ pump in inflamed chondrocytes and the K^+/Na^+ ratio in faeces, a sample can be used as a diagnostic tool for lameness in horses.

Increased degree of movement asymmetry may be an indicator of OA, because the pain associated with the disease might cause the horse to change motion pattern (Baxter *et al.*, 2011). Horses usually try to move in a motion pattern that creates

the least pain, therefore the symmetry might be changed (Baxter *et al.*, 2011). Lameness in horses is complex and diagnosing is not always easy, even for the most experienced veterinarians. Lameness Locator® is a wireless, sensor-based motion analysis system which can be used to objectively measure symmetries and asymmetries in motion pattern in horses (Keegan *et al*., 2011).

Today classical radiographic examinations are used to diagnose OA, however in later years focus has been shifted towards biochemical biomarkers (Baxter *et al*., 2011). OA assays have been developed that detect matrix molecules, but these markers are not sensitive enough for individual diagnostics since they reflect both degradation and synthesis of the matrix as well as an overall inflammation. The existing biomarkers cannot be used for early diagnose of OA and thus not paving the road towards new treatments (Baxter *et al*., 2011).

It would be desirable to find a biomarker for early stages of OA to be able to start treatment before irreversible damage to articular cartilage has occurred (Skiöldebrand *et al*., 2018).

1.1 Hypothesis

The hypothesis was that horses with confirmed pain induced lameness have a high K^+/Na^+ ratio in faeces, due to an altered function of the Na^+/K^+ pump in inflamed chondrocytes in articular cartilage associated with osteoarthritis. Faecal K^+/Na^+ ratio could then be used as a biomarker for osteoarthritis.

1.2 Objective

The objective of this study was to determine whether there is a correlation between the faecal K^+/Na^+ ratio and lameness in horses associated with osteoarthritis.

2 Literature review

Osteoarthritis (OA), a chronic joint disease with low-grade inflammation, is the most common cause for joint pain and lameness in horses (Perkins *et al*., 2002). Many horses retire at a young age due to lameness which is the most common reason for failure to train and race (Perkins *et al*., 2002). OA, the major disease behind chronic joint pain with subsequent clinical lameness, accounts for an important animal welfare concern, as well as being the greatest single economic loss for the horse industry (van Weeren & de Grauw, 2010).

The need for a biomarker to detect the destructive painful and progressive processes in the joint on an individual level is urgent (Kamm *et al*., 2010). Imaging grading has been the traditional marker in studies involving diagnosis and progression of the lesions present in the OA joint. The different imaging techniques available today can only evaluate the irreversible morphological changes and not the early catabolic biochemical processes (Kamm *et al*., 2010). The OA disease includes a low grade inflammation with pro-inflammatory cytokines; interleukin-1 β (IL-1 β) and tumor necrosis factor (TNF)-α present in the early stages (Goldring *et al*., 2011). Activity of IL-1 β has been detected in synovial fluid from equine OA joints and has been shown to induce gene expression of matrix degrading MMP:s (matrix metallopeptidases) and ADAMTS (a distintegrin-like and metalloproteinase with thrombospondin type 1 motif) in chondrocytes. (Kamm *et al*., 2010).

Chondrocytes are responsible for synthesis, assembly and turnover of the extracellular matrix (ECM) constituents, where mechanical load is one important stimulus for chondrocyte metabolism (Heinegard & Saxne, 2011). Assays have been developed that detect matrix components like cartilage oligomeric matrix proteins (native molecule of COMP), and inflammatory cytokines in synovial fluids or serum from horses with OA. However, these markers are reflecting both degradation and synthesis of the matrix as well as an overall inflammation. Today

the commercial available assays cannot detect early OA lesions in a joint on an individual level and it is impossible to distinguish pathologic cartilage fragmentation from normal turnover (Heinegard & Saxne, 2011).

The ECM of the cartilage is mainly composed of aggrecan and collagen type II, providing elasticity and contributing to tensile strength, respectively (Svala *et al*., 2015). Other macromolecules, such as; COMP, fibronectin, fibromodulin, chondroadherin, biglycan, decorin are also present and involved in the assembly and interactions of different molecular networks as well as with the chondrocytes (Svala *et al*., 2015). Normally the cartilage is actively remodelled to adapt the joint to different load, with a balance in degradation and synthesis of matrix proteins (Salter *et al*., 2002). Loss of joint function is associated with proteolytic destruction of aggrecan and the collagen networks with subsequent impaired articular cartilage matrix (Svala *et al*., 2015). Unique fragments, only present in diseased cartilage are formed during this matrix destruction in IL-β stimulated cartilage explants and in equine disease (Svala *et al*., 2015).

The early tissue inflammation in osteoarthritis alters several biochemical parameters such as cellular swelling, microfilament reorganization and impaired ion exchange of the chondrocytes in the articular cartilage (Skiöldebrand *et al*., 2018). Ca^{2+} signalling within the cell and between cells is very important for the cellular homeostasis. In the case of inflammation, the sodium and potassium exchange of chondrocytes will affect the intracellular Ca^{2+} release followed by excessive extracellular cytokine release (Skiöldebrand *et al*., 2018).

2.1 Na^+/K^+ pump in the cell membrane

The Na^+/K^+ pump (Na^+/K^+-ATPase, or sodium-potassium pump) is an enzyme located in the plasma membrane in all cells (figure 1) (Sjaastad *et al*., 2003). Its function is to move sodium out of the cell, and potassium into the cell, as to maintain the membrane potential by having a higher sodium concentration outside of the cell, and a higher potassium concentration inside the cell. As both these transports take place against the concentration gradients, the pump is active and uses energy in form of ATP (adenosine triphosphate). In one cycle of action three sodium ions are moved out of the cell and two potassium ions into the cell. This uses the energy of one ATP changing into ADP (adenosine diphosphate). Two important roles of the Na^+/K^+-pump are to maintain cellular resting potential and to regulate cellular volume by affecting osmosis of water (Sjaastad *et al*., 2003).

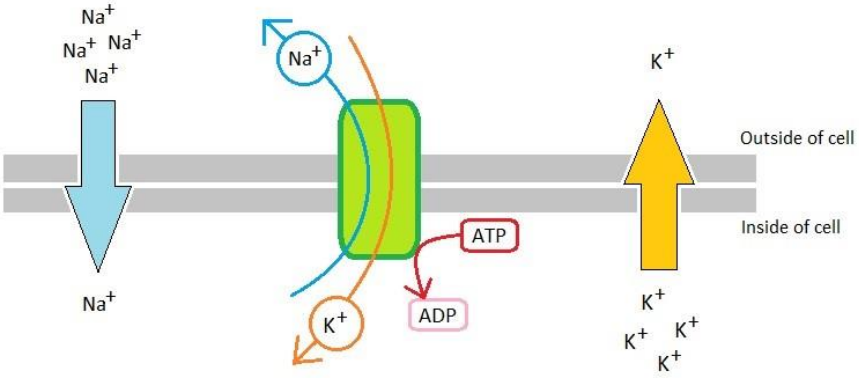

Figure 1. The $Na^{+/}$ K^{+} pump. The arrows show the electrochemical concentration gradient for potassium and sodium. (After Alberts *et al*. 2009)

The Na^{+}/K^{+} pump also affects, to an extent, transportation of Ca^{2+} (Sjaastad *et al*., 2003). The Na^{+}- Ca^{2+}-exchanger is a membrane protein working as an antiporter. It is moving Ca^{2+} out of the cell (against the concentration gradient) and creates energy to this transport by letting Na^{+} flow into the cell (down the concentration gradient). The transport of one Ca^{2+}requires three Na^{+}. Apart from the Na^{+}- Ca^{2+}-exchanger, the plasma membrane Ca^{2+}-ATPase also contributes to extracellular transport of Ca^{2+} (Sjaastad *et al*., 2003). Therefore, the action of the Na^{+}/K^{+}-pump indirectly affects Ca^{2+} signalling (Skiöldebrand *et al*., 2018) Inflammation downregulate this pump and therefore Ca^{2+} homeostasis in the cell is changed, which will affect the chondrocytes homeostasis negatively (Skiöldebrand *et al*., 2018).

2.2 Faecal K^{+}/Na^{+} ratio

Jansson (1999) reported changes in K^{+}/Na^{+} ratio in faeces 3-6 hours after training and treatment with aldosterone and therefore suggested faecal samples as a good indicator of sodium status. Also, according to a study of Jansson *et al*. (2010), faecal K^{+}/Na^{+} ratio can be used as an indicator of sodium status. K^{+}/Na^{+} ratio is, in comparison with Na^{+}/K^{+} ratio, more affected by the intake of sodium than individual differences. Horses with a ratio >10 may considered to have a diet low on sodium (Jansson *et al*. 2010).

2.3 Potassium

Potassium (K) is involved in the maintenance of the acid-base balance and osmotic pressure (Frape, 2010; Sjaastad *et al*., 2003). Potassium is also the most quantitatively important ion for neuromuscular excitability (Frape, 2010; Sjaastad *et al*., 2003). Potassium is mainly absorbed before caecum and the digestibility is approximately 75% (Frape, 2010). Excretion of the excess occurs via the kidneys. Sweat from the horse contains 1,4 g K/L (Frape, 2010).

2.3.1 Maintenance

The maintenance, to achieve equilibrium, for potassium in an adult horse is 48 mg/kg BW and day, while the maintenance with addition of moderate exercise is 64 mg/kg BW and day (Frape, 2010). Calculations for exercise is based on a loss of 2,8 g potassium/kg BW in sweat, and a loss of bodyweight of 10-20 g/kg BW (Frape, 2010).

Pasture contain large amounts of potassium in the dry matter and hay contains 15-25 g K/kg (Frape, 2010). Cereals are low in potassium content. Most horses are supplied with enough potassium if the diet consists of at least one-third hay of good quality. If horses in hard training are fed with larger amounts of cereals, this would lower their intake of potassium, at the same time as losses in sweat would normally be increasing (Frape, 2010).

2.3.2 Deficiency and excess

In case of potassium deficiency in the diet the horse can develop reduced appetite, lower growth rate and decreased plasma K (hypokalaemia) (Frape, 2010). In extreme deficiency, clinical muscular dystrophy and stiffness of the joints occurs. (Frape, 2010).

Excessive potassium is regulated first by increased excretion to the urine, and then to the faeces (Frape, 2010). Jansson (1999) found no retention of potassium when intake exceeded 210 mg/kg BW. Increased water intake and urine volume was noted (Jansson, 1999). A maximum tolerance level of potassium has not been seen when the horse has access to water (Frape, 2010).

2.4 Sodium

Sodium (Na) is an important determinant of the osmolarity of extracellular fluid and the volume of that fluid (Frape, 2010; Sjaastad *et al*., 2003). Chloride concentration is directly related to that of sodium. Sodium is also of importance for the normal function of the central nervous system, generation of action potentials in excitable tissues and transport of many substances, for example glucose, across cell membranes (Frape, 2010; Sjaastad *et al*., 2003). Sodium is the most important extracellular cation (138-140 mmol/L) and the major electrolyte for the maintenance of acid-base balance and osmotic regulation of body fluids (Frape, 2010).

Approximately 95% of the sodium is normally absorbed in the large intestine, but in the case of sodium deficiency the absorption can increase up to 99% and renal excretion is reduced (Frape, 2010). The kidneys control the secretion of sodium and is controlled by the renin-angiotensin-aldosterone system. Sweat from the horse contains 2,8 g Na/L. During a severe deficiency the composition of sodium and potassium concentrations in sweat is changed by having a decrease in plasma sodium (up to 120 mmol/L) and chloride (70 mmol/L), increase in plasma potassium (up to 5.5 mmol/L) and a decrease in total body water, mostly because of increased dry matter of the gastrointestinal contents. Grass, hay and haylage often contain less than 0,1% sodium and cereals content are even lower (Frape, 2010).

2.4.1 Maintenance

The maintenance, to achieve equilibrium, for sodium in an adult horse is 20 mg/kg BW and day, while the maintenance with addition of moderate exercise is 51 mg/kg BW and day (Frape, 2010). The numbers for maintenance for exercise assume that 3,1 g Na is required/kg BW lost as sweat during exercise and that 10-20 g BW is lost per kg BW during exercise (Frape, 2010).

Horses can to some extent adapt to low sodium content in the diet, but horses in hard training may need supplementary salt because of increased sweating (Frape, 2010). Since the concentration of sodium and potassium is adjusted by aldosterone in the kidneys, diets with 2-4 g Na/kg should meet the need, except when increased sweating in hot weather occur (Frape, 2010).

In a study of Jansson and Dahlbom (1999), horses in training with only access to a lick stone was found to have a sodium intake exactly, or lower than, the daily

maintenance. Therefore, these horses are recommended to be given a supplementary intake of salt (Jansson & Dahlbom, 1999).

2.4.2 Deficiency and excess

Signs of sodium deficiency include an increase in cutaneous turgor, the quest for licking salty objects, decreased water intake and a slow rate and/or cessation of eating (Frape, 2010). Sodium deficiency might also cause a craving for sodium chloride and muscular and nervous dysfunction (muscular tremor, gait and chewing incoordination) (Frape, 2010).

Aldosterone is a hormone formed in the adrenal cortex and regulates the body's secretion of sodium and potassium. (Sjaastad *et al*., 2003). By saving sodium, to save water, aldosterone maintains/increases blood pressure. The increase in plasma aldosterone level causes increased sodium absorption (Sjaastad *et al*., 2003). Endogenous and exogenous aldosterone does affect the fluid balance regulation in the horse by reducing sodium losses in urine and faeces, both at rest and after exercise (Jansson, 1999). Hyponatremia can be seen in horses with reduced intake of sodium, this will cause aldosterone secretion (Frape, 2010; Sjaastad *et al*., 2003). Sodium deficits can also occur through losses from the gastrointestinal or urinary tract relative to water loss (Frape, 2010; Sjaastad *et al*., 2003).

According to a study of Jansson *et al*. (2010), an increase in plasma aldosterone concentration was seen in direct connection to training in a group of horses on a diet low in sodium. Seven standardbred geldings in training (twice a week) were offered a diet supplemented or non-supplemented with NaCl for five weeks. Blood samples were taken once a week and during week five; before and after training until 22h30. Lower levels in blood sodium concentration was seen in samples 15 minutes after training and in the evening. Horses supplied with 3 mg Na/kg BW had still lower blood sodium content than horses supplemented with 58 mg NA/kg BW 7,5-13,5 h after training (22h30). Horses supplemented with NaCl were 19h30 back to same plasma aldosterone levels as before training, while the group of horses with a diet low in sodium still had high levels 22h30 (Jansson *et al*. 2010).

2.5 Chloride

When the requirements for sodium are fulfilled with salt (NaCl), the requirements for chloride will also be met (Frape, 2010; Sjaastad *et al*., 2003). Deficiency is

unlikely, but if horses are given large amounts of sodium bicarbonate it would be possible (Frape, 2010; Sjaastad *et al*., 2003).

2.6 Definitions of movement, asymmetry and unsoundness

Movement describes the horses' travel and action (Baxter *et al*., 2011). Travel is defined as the path of the hoof (limb) flight in relation to the midline of the other limbs, whilst action is the style of movement, including joint flexion, stride length and suspension. Asymmetry is defined as a difference between two body parts or an alteration in the synchronization of a gait. A horse can be considered "unsound" if it is lame, has limited performance or is otherwise unserviceable. Evenness is defined as a horse in balance, symmetry and with synchronization of the steps within a gait with correct weight bearing and timing (Baxter *et al*., 2011).

2.7 Lameness

Lameness is an indication of a structural or functional disorder in one or more limbs or the back that is evident while the horse is standing or at movement (Baxter *et al*., 2011). Lameness can be caused by several reasons, or any combination of them. Horses adapt to lameness with compensatory movements of other body parts when using the lame limb (Baxter *et al*., 2011).

2.8 Clinical evaluation of lameness

2.8.1 Subjective motion assessment

Before motion assessment was possible to perform with any kind of kinematics, the veterinarian had to rely on his own eyes in a subjective assessment (Baxter & Stashak, 2011). For veterinarians worldwide, this is still the most widely used method (Baxter & Stashak, 2011).

Flexion test is a clinical method to exacerbate subtle lameness and is commonly used by veterinarians in Sweden (Baxter & Stashak, 2011). Flexion tests is also used in trying to determine from which joint or structure in the leg the lameness could origin. The limb and joints are flexed, and pressure is held for 30 to 60 seconds. The horse is evaluated in trot at straight line immediately after the pressure has been released and any lameness is registered. Although flexion tests have a high variability, for example small differences in the way the veterinarian

hold the limb or apply the pressure in the limb, the technique is usually enough to objectively assess responses to flexion when performed by an experienced veterinarian (Baxter & Stashak, 2011).

To localize the painful area in a lame limb, local anaesthesia is commonly used during a lameness examination (Baxter & Stashak, 2011). A local anaesthetic is injected into the joint. After allowing for onset of action, the horse is again evaluated for lameness and flexion tests are repeated (Baxter & Stashak, 2011). One challenge with intra-articular anaesthesia is that the anaesthetic agent can diffuse out of the joint so that anaesthesia of periarticular structures can occur, making the diagnosis more difficult. (Gough *et al*., 2002a; Gough *et al*., 2002b). As with all intra-articular injections there is a risk of iatrogenic infection with development of septic arthritis, even if the incidence is low (Steel *et al*., 2013). As an alternative to intra-articular anaesthesia nerve blocks can be performed (Seabaugh *et al*., 2011). The local anaesthetic is injected adjacent to a nerve resulting in a total anaesthesia distal to the block. The nerve block is less specific in diagnosing articular lameness (Seabaugh *et al*., 2011).

2.9 Kinematics at trot

One of the horse's gaits, footfall patterns, is trot (Baxter *et al.*, 2011). Trot is a two-beat diagonal gait. Left fore and right hind moves together when right fore and left hind is on the ground (Baxter *et al.*, 2011). Head and pelvis are at its highest point in the swing phase and lowest in the middle of the stance phase, for both diagonals (Santesson *et al.*, 2017).

Measurement and study of movement is called kinematics (Keegan, 2011). Left and right-side differences in symmetry are measured when using kinematics as an objective assessment of lameness in horses (Keegan, 2011). Asymmetry occurs because the horse puts less weight on the lame leg and the head respective the pelvic region is not lowered as much compared to the sound leg (Baxter *et al*., 2011). Therefore, the difference between the two body parts occurs (Baxter *et al*., 2011). If the horse is equally lame in both left and right limbs, the measurement will not show asymmetry between the left and right strides (Keegan, 2011). Asymmetric motion of the torso, head, neck and limbs may also occur for many other reasons than lameness. Conformational disparity between left and right limbs and preference to use one limb over the other will affect the symmetry (Keegan, 2011).

2.9.1 Measurement of locomotion asymmetry

A body-mounted wireless, inertial sensor-based motion analysis system can be used to measure symmetries/asymmetries in motion patterns (Keegan *et al*., 2011). According to Equinosis (2017) Lameness Locator® is a real-time, handheld, field-based system that enables one to objectively measure locomotion symmetry in horses with non-invasive inertial sensors (figure 2) (Keegan, 2011). Lameness Locator® was designed as an easy aid for detection and evaluation of difficult lameness but is nowadays mostly used to detect mild lameness where the human eye has difficulties detecting fast events in the movement (Keegan, 2011).

Figure 2. Horse equipped with an accelerometer on the head and the pelvic region, and a gyroscope on the right front leg during a measurement with Lameness Locator®. (Photo: Haide Westring)

Lameness Locator® shows the difference between vertical maximum and minimum head and pelvic movements (Keegan *et al*., 2011). The horse is equipped with uniaxial accelerometers (Keegan *et al*., 2011).

Hard level surface is best suited for the assessment of symmetry since this provides more load than a softer surface (Keegan, 2011). The handler affects the measurement and need to try to hold the horse loosely with its head centred in line with its body. The distance should be performed in a straight line as slowly as practical. Movement of the head and neck from side to side often results in an asymmetric gait (Keegan, 2011).

Head and pelvis are nodding respective lowered twice per cycle, since trot is a two-beat gait (Keegan, 2011). Two maximum respective two minimum values are

registered per cycle, which is illustrated in a symmetric curve (figure 3). Asymmetry is quantified by the difference in maximum head or pelvic positions between left and right limb strides (diff max head/pelvis) and the difference between minimum head/pelvic position between left and right limb strides (diff min head/pelvis) (Keegan, 2011).

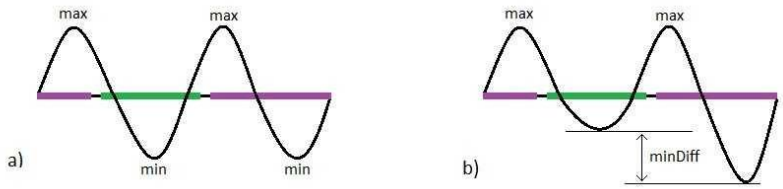

Figure 3. Symmetric (a) and asymmetric (b) curves representing the head or pelvic movements per stride cycle. (After Santesson *et al*., 2017)

Thresholds by the Lameness Locator manufacturer (Equinosis® Q) for head (front limb lameness) diff max and head diff min is ±6 mm (Keegan, 2011). Standard deviations of head diff max and mean less than mean head diff max respective min, indicates lameness or could at least be considered as asymmetry. The threshold between hind limb symmetry and asymmetry determined by the Lameness Locator manufacturer (Equinosis® Q) is ±3 mm (Keegan, 2011).

Locomotion symmetry was studied within a group of sixteen Standardbred horses for 2,5 years, from their start in September as one-year-olds, until they had reached the age of three (Ringmark *et al*., 2015). The values were stabile until spring as two-year-olds, when the horses were introduced to speed training. Thresholds were defined during the stabile period, from the autumn as one-year-olds. The thresholds were defined to 8,5 for vector sum (VS) front respectively 4,0 for VS hind (Ringmark *et al*., 2015).

According to a study by Rhodin *et al.* (2017), 72,5% of the horses considered sound by their owners, showed motion asymmetries above those defined by Keegan (2011) at straight line trot. Vertical head and pelvic movement symmetry were measured with Lameness Locator® in 222 warmblood type riding horses. It is also emphasized that it is not known to what extent these asymmetries are related to pain or to mechanical abnormalities (Rhodin *et al.*, 2017).

3 Materials and methods

3.1 Horses and study design

Data collection started in September 2017 and ended in May 2018. In total 62 horses assessed as sound by their owners, were subjected to locomotion analysis (Lameness Locator®, Equinosis® Q). The objective was to include a group of 15 horses, symmetric enough for the horse to be considered as not likely affected by joint OA (non-lame). Horses included had not been treated pharmaceutically within 6 months prior to enrolment in the study. The horses were found through personal contacts and the 62 horses were located at eleven different stables. The locations and number of stables were as follows; Knivsta (2), Kolbäck (2), Upplands Väsby (5), Uppsala (1) and Årsta (1). All horses were in light to moderate training, as defined as it being ridden at least three times a week. Four horses were excluded due to lameness and one was sold at the time for the second measurement.

In total 27 horses diagnosed as lame were included at the University Animal Hospital, Uppsala (in December 2017, n=6) and at Brunmåla Horse Clinic in Täby (in December 2017, January and May 2018, n=21). Horses included had the following characteristics; considered to show pain induced lameness according to the clinical assessment and lameness examination including flexion test and intra-articular anaesthesia with or without radiological changes in one or several joints.

None of the horses included in the two groups were in hard training the day before the measurement respectively the visit at the clinic.

3.2 Assessment of locomotion symmetry in sound horses

Locomotion symmetry was assessed twice, with four to six weeks in-between. The horses were assessed in-hand on soft (riding arena) and hard surface (frozen surface in paddock or gravel surface) and on the lunge on both reins on soft surface (riding arena). From the selectable surfaces in Lameness Locator®, soft deep and packed dirt were selected to correspond the chosen surfaces. The assessment consisted of straight line at trot for about 60 meters back and forwards and for the lunging; circles with a diameter of 18 meters. The aim was to include horses with at least 25 continuous strides, in line with recommendations from Keegan (2011). Measurements from straight line on hard surface was finally included in the study. The measurements were done by the author.

3.2.1 Locomotion symmetry

Data from Lameness Locator® Software (Equinosis® Q) was exported to Microsoft Excel (2010). Horses with only a single measurement were excluded. Data was cleared by trial and surface; only straight line on hard ground (packed dirt and asphalt) was remained. Thereafter data was cleared by number of strides, horses with at least 20 continuous strides were included. In cases where the horse had several measurements in the same occasion, the last was chosen.

From the differences between movements of head and pelvis the vector sum (VS) was calculated as;

$$VS = \sqrt{max\,difference^2 + min\,difference^2}$$

This was done both for head and pelvic sensors (VS front and VS hind). Thresholds for symmetry were defined to 9,0 for VS front and 4,5 for VS hind, 0,5 from the thresholds defined by Ringmark *et al.* (2015).

3.2.2 Lameness evaluation

The horses were defined as lame when having a positive result (increased lameness) at a flexion test and reduced lameness with intra-articular anaesthesia of at least one joint. No objective lameness assessment tool was used. Both horses with and without radiological changes of the joint were included.

3.3 Faecal sampling and analysis of faecal K^+/Na^+ concentration

Faecal samples of at least 100 grams was collected from each horse. Collection of samples was made after the second measurement for the group of symmetrical horses, and in conjunction with the clinic visit for the lame horses. All samples were collected within maximum 24 hours after the measurement respective the visit at the clinic and stored frozen.

The analysis of faecal concentration of Na^+ and K^+ was performed by AgriLab, Uppsala, Sweden. The frozen samples were first thawed and weighed. The samples were thereafter dried in a climate cabinet (Termaks TS8056, AB Ninolab) at 105°C for at least 24 hours. After drying, the samples were weighed again for calculation of dry matter. The dried samples were pulverized and 1 gram of faecal powder was blended with 20 ml of nitric acid (HNO_3, 7 M, Sigma-Aldrich, Nitric acid puriss. p.a., reag. ISO, reag. Ph. Eur., for determinations with dithizone, ≥65%). Next step for the samples were heating to 100°C for 1 hour and then deionized distilled water was added to each sample to a total volume of 100 ml. After that a tenfold dilution was made using deionized distilled water. The diluted liquid was used to determine the concentration of Na^+ and K^+ by ICP-OES (Spectro Blue FMS26). Measured values were adjusted according to the exact inverse amount. A ratio for K^+/Na^+ was calculated from the given values.

3.4 Data collection

Horse owners who, upon request, accepted to participate in the study responded orally to questions about the horses' age, training status and access to salt. The horse owners were asked if they gave salt supplements every day (at least 1 tablespoon NaCl/day) to the horse or not.

3.5 Statistical analysis

The data was analysed using GraphPad Prism version 5.01 for Windows (GraphPad Software). The data is expressed as mean and standard deviation (± SD). Statistical difference between sample groups were assessed using Students unpaired t-test. A significant difference was assumed at a p-value of ≤0,05.

4 Results

4.1 Symmetric and lame horses

In total nine horses were considered symmetric. When using thresholds for VS defined by Ringmark *et al.* (2015) seven horses met the criteria. They were in-between the thresholds for both VS front and hind during both measurements. Two additional horses were included, because they were well beneath the threshold for VS front during both measurements, but had VS hind slightly above 4,0 (4,1 respective 4,2) during one of the measurements.

The mean (±SD) age of the symmetric horses was 10,2 (±5,0) years. The mean (±SD) vector sum fore for both measurements was 4,8 (±2,5) and the vector sum hind for both measurements was 2,4 (±1,2).

The mean (±SD) age of the lame horses at the clinics was 11,1 (± 3,8) years.

4.2 K^+/Na^+ ratio in faeces

The mean (±SD) K^+/Na^+ ratio for the symmetric horses was 8,4 (±8,8) and for the lame horses 8,9 (±24,4). No statistical difference was found between the groups (figure 4). The mean (±SD) concentration of K^+ for symmetric horses was 10,3 (±2,8) and for the lame horses 10,6 (±3,2). No statistical difference was found between the groups (figure 5). The mean (±SD) concentration of Na^+ for symmetric horses was 2,6 (±2,1) and for the lame horses 4,0 (±3,0). No statistical difference was found between the groups (figure 6).

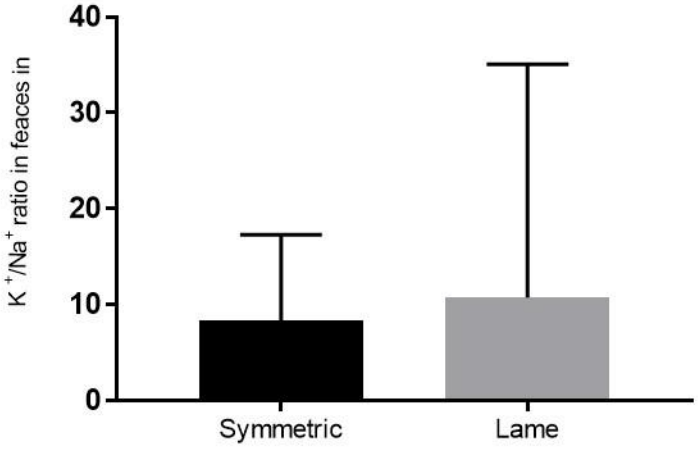

Figure 4. The mean K^+/Na^+ ratio in faeces for symmetric and lame horses.

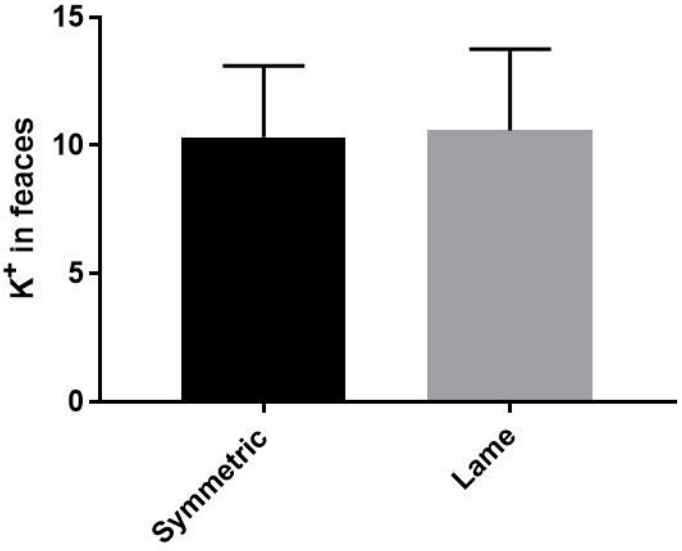

Figure 5. The mean concentration of K^+ in faeces for symmetric and lame horses.

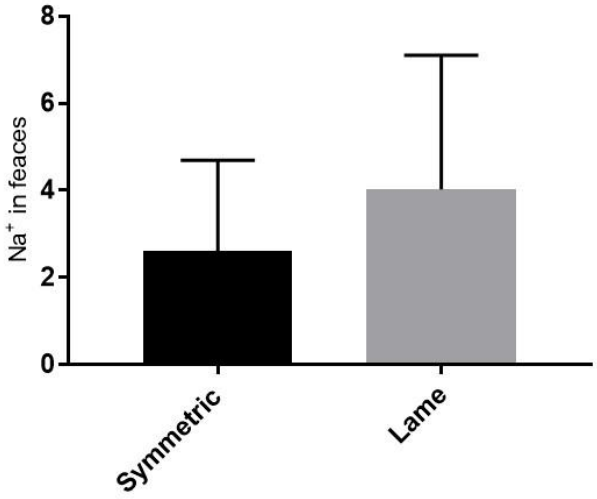

Figure 6. The mean concentration of Na^+ in faeces for symmetric and lame horses.

4.3 Salt supplementation

For the symmetric horses three were supplemented daily with salt, five not and one horse-owner did not respond to the question. For the lame horses seven horses were supplemented daily with salt and 20 were not.

The mean K^+/N^+ ratio in faeces for horses (n=10) that received salt supplement daily in the feed was 4,7 (±26,5) and the horses (n=23) that not received any supplementation was 13,2 (±5,0). No statistical difference was found between the groups (figure 7).

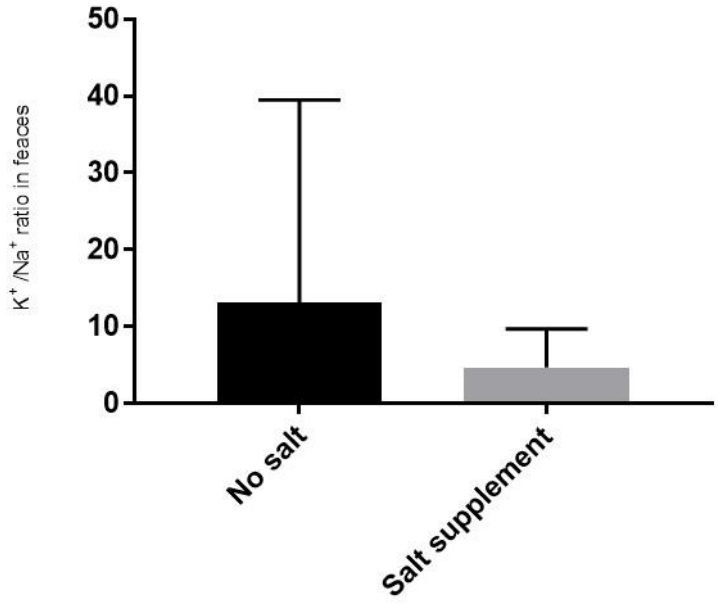

Figure 7. The mean K^+/Na^+ ratio in faeces for horses supplemented daily, or not, with salt in the feed.

5 Discussion

Osteoarthritis (OA) is a common disease with lameness due to joint pain as the major characteristic. OA is a chronic low-grade inflammation and affects the entire joint. The chondrocytes react to the inflammation with a change in cellular parameters such as Ca^{2+} signalling, downregulation of extracellular matrix protein and impaired K^+/Na^+ exchange. The aim of the study was to determine if a high ratio of faecal K^+/Na^+ could serve as a biomarker for lameness associated with OA. In this age-matched study no statistical differences were found between symmetric and lame horses, why faecal K^+/Na^+ ratio cannot be recommended as a biomarker for lameness in horses with OA.

The lame horses were included by lameness examination and intraarticular anaesthesia, and no further diagnostic tests (i.e. radiographic examinations, synovial sampling) were done. Therefore, it cannot be ruled out that some horses were lame due to other reasons and that may be affecting the results. Additionally, the lameness duration was not recorded and that could also be an important parameter.

To ensure that the horse was sampled during a period when it was symmetric, the group of horses were followed over a period of four to six weeks. The faecal sample was taken in conjunction with the second measurement, to represent the period between the two measurements. The faecal samples from the lame horses were taken in conjunction with the visit at the veterinary clinic and may be considered to represent a period of lameness. It was ensured that the horses included had not been in hard training the day before faecal sampling, since this could affect the result (Jansson *et al.* 2010).

Several of the lame horses included in this study was on a revisit to the clinic and had therefore been considered lame for a period of at least approximately three weeks, since the first visit. In some cases, the horses had been lame for an even

longer period, up to six months, so the group with lame horses represents both acute and chronic lameness. There were no significant differences in faecal K^+/Na^+ ratio between lame and symmetric horses, this might have been different with other selection criteria.

Salt supplementation could affect faecal K/Na-ratio and alter the result. As part of the study, it was recorded if the horse was provided with supplementary salt or not. However, the exact amount of supplemented salt or the total amount in the horses' feed was not registered. Even though there were no statistically significant difference, the result indicates that horses not daily supplemented with salt had a faecal K^+/Na^+ ratio slightly above the ratio earlier reported as high (Jansson *et al*., 2010). This indicates that only access to a lick stone is not always sufficient, even for horses in light training. This is earlier reported for horses in hard training (Jansson & Dahlbom, 1999).

A challenge in this study was the definition of sound horses. The Lameness Locator® was chosen as an objective way to measure motion patterns. Alternative ways could be to include horses considered sound at for example a pre-purchase examination, by questionnaire to owners asking about the horses' history of lameness and training or to obtain data from insurance companies about horses without reported lameness history. These other methods might have had other advantages and disadvantages.

Asymmetry is defined as a difference between two body parts or an alteration in the synchronization of a gait by Baxter *et al.* (2011), therefore the assumption was that symmetric horses correspond to non-lame horses. The aim was to define a horse with a trot on a straight line symmetric enough for the horse to be considered not likely affected by joint arthritis. Initially, the plan was to include fifteen lame and fifteen symmetric horses. However, most of the horses had symmetric values above the earlier defined thresholds for vertical maximum and minimum head and pelvic heights between left and right forelimb and hindlimb stances measured with Lameness Locator® (Keegan 2011). To include the needed number of symmetric horses, the thresholds had to be changed, because a large part of the measured horses had results above earlier defined thresholds. In line with Rhodin *et al*. (2017) only a small number of horses were symmetric according to the Lameness Locator®, although all measured horses were perceived as sound and healthy by their owners and riders.

The number of horses in this study is small and the reason why there is a big difference in the number of symmetric (n=9) and lame (n=27) horses, was the

difficulty of finding symmetric horses. Even when including measurements from both hard and soft ground, very few horses were within the thresholds during both measurements. When changing criteria to VS front and hind, measured on hard ground, seven horses were found within thresholds defined by Ringmark *et al*. (2015). One strength with this study is that the horses selected as the most symmetrical out of 62 horses, were chosen for their absolute values, neither mean values or by picking out the fifteen most symmetric. The decision to include the two horses that were slightly above 4,0 for VS hind (4,1 respective 4,2) seemed reasonable to bring up the numbers of symmetric horses, since they were well beneath the threshold for VS front and their first respective second measurement were below the threshold.

To be able to refer to Ringmark *et al.* (2015), straight-line trot on hard ground was chosen. However, bilateral lameness, equal on both sides, can be difficult to reveal from straight line trot. This could be one reason to include also measurements on circle.

Since horses adapt to lameness to unload the lame limb during weight-bearing or stance phase of the stride (Baxter *et al.*, 2011), it is likely that the nine chosen symmetric horses were sound. However, because measurements were made only on straight line, it cannot be excluded that horses with bilateral asymmetries were included. To reduce the risk of choosing lame horses, an objective kinematic measurement could be combined with a clinical assessment including a flexion test.

The horses and horse owners should maybe have been given a period of acclimatization respective training before the Lameness Locator® examination. Some of the horses might react, more or less, to the equipment. A drawback is that the measurements are also affected by the handler (Keegan, 2011). All owners were, when needed, encouraged to run with a loose contact in the reins or lead rope and the head of the horse centred in line with the body. Another difficulty for the handler was to run in a straight line. An alternative could have been to have the same handler during all measurements. External environmental impact might decrease with several trials included.

As shown in earlier studies, a large proportion of horses in training, considered sound by their owners, show motion asymmetries (Rhodin *et al*., 2017). The same was shown in this study where all the 62 measured horses were considered sound and well-functioning in riding by their owners, but only nine met the thresholds. Rhodin *et al.* (2017) also clarifies that it is not clear to what extent asymmetries

are related to an underlying disease and if horses with asymmetries always are subject to a welfare problem.

When performing the measurements, subjectively, the horses with more expressive gaits appeared to show more asymmetry. Many modern sport horses are hypermobile and further research in how this affect the motion pattern is needed. To some extent the asymmetries might be biological variation and not pain induced. Another question is how the symmetry/asymmetry will change with age and education of the horse.

5.1 Conclusion

In this study no correlation was found between faecal K^+/Na^+ ratio and joint related lameness in horses. Therefore, faecal K^+/Na^+ ratio is not a suitable biomarker for early OA.

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