University of Veterinary Medicine, Budapest

Institute of Animal Breeding, Nutrition and Laboratory Animal Science

Department of Animal Nutrition and Clinical Dietetics

Review of oral joint supplementation for horses sold on the internet: analysis of provided information and scientific evidence

Tamara Abela

Supervisor: Dr. Moravszki Letícia, Department of Animal Nutrition and Dietetics

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List of abbreviations

ECM: extracellular matrix

OA: osteoarthritis

IL: interleukin

PGE2: prostaglandin E2

OJSs: oral joint supplements

NADG: N-acetyl-D-glucosamine

GAGs: glycosaminoglycans

COX: cyclooxygenase

CS: chondroitin sulfate

NF-κB: nuclear factor kappa beta

5-LOX: 5-lipoxygenase

ASU: avocado soybean unsaponifiables (ASU)

ARA: arachidonic acid

PG: prostaglandins

SOD: superoxide dismutase

GPx: glutathione peroxidase

TNF-α:tumour necrosis factor alpha

LOX: lipoxygenase

IFN-γ: interferon gamma

LA: lipoic acid

KBA: keto-β-boswellic acid

AKBA: 3-O-acetyl-11-keto-β-boswellic acid

α-BA: alpha boswellic acid

β-BA: beta boswellic acid

α-ABA: 3-O-acetyl-α-boswellic acid

β-ABA: 3-O- acetyl-β-boswellic acid

MMP: matrix metalloproteinase

FAs: fatty acids

ALA: alpha linoleic acid

EPA: eicosapetaenoic acid

DPA: docosapentaenoic acid

DHA: docosahexaenoic acid

ώ-3LCPUFA: omega-3 long chain polyunsaturated FAs

NSAIDs: non-steroidal anti-inflammatory drugs

HA: hyaluronic acid

PK: phylloquinone

MK-n: menaquinone

MD: menadione

C2C: collagenase cleavage neopeptide

CPII: carboxypeptide of type II collagen

UC-II: undenatured type II collagen

Treg cells: T regulatory cells

Si: silicon

SC: saccharomyces cerevisiae

BG: beta-glucan

SM: silymarin

NEM: natural eggshell membrane

Cu: copper

Zn: zinc

Mn: manganese

Co: cobalt

I: iodine

Mg: magnesium

AAEP: American Association of Equine Practitioners

Abstract

Abstract in English

Oral joint supplements are very popular with horse owners since problems with the locomotor system are a common cause of why sport horses retire. Oral joint supplements are claimed to possess anti-inflammatory, anti-oxidative, chondroprotective and cartilage building properties. Due to these properties, owners use these supplements to improve performance, prevent and/or eliminate problems. However, even though manufacturers have increased the production of these products which can be easily bought, it still difficult to determine the efficacy of these supplements. The aim of this literature review was to therefore determine whether these claimed effects have been proven *in vivo* in equine. Results demonstrated that, in most cases further research is necessary. The ingredient lists of the products were also evaluated to check whether they are complete. Results demonstrated that this was not the case for some of the products.

Abstract in Hungarian

A szájon át adható ízületvédő takarmány kiegészítők nagyon népszerűek a lótartók körében, mivel a mozgásszervi megbetegedések az egyik leggyakoribb okai a sportlovak idő előtti visszavonultatásának. Ezeknek a kiegészítőknek gyakran tulajdonítanak gyulladáscsökkentő, antioxidáns, porcvédő és porcépítő tulajdonságokat, melyeknek köszönhetően a tulajdonosok ezeket a teljesítmény javítására, a problémák megelőzésére és/vagy megszüntetésére használják. Mivel ezek a termékek könnyen hozzáférhetőek az állattartók számára, így a gyártók folyamatosan növelik a forgalomba hozott termékek körét, annak ellenére, hogy még mindig nehéz meghatározni a hatékonyságukat. Ezen irodalmi áttekintésnek az a célja, hogy megállapítsa, hogy ezeket az állítólagos hatásokat megerősítették-e lovakon végzett *in vivo* vizsgálatokkal. Az eredmények azt mutatják, hogy az esetek többségében további kutatásokra lenne még szükség. A termékek összetevőinek listája is kiértékelésre került, és az eredmények azt mutatták, hogy egyes termékek esetében az nem volt teljes.

1. Introduction

One of the most frequent causes of poor performance and early retirement of sport horses is the impaired function of the locomotor system. Osteoarthritis can cause lamness, but other factors, such as damaged tendons, ligaments and muscles can lead to joint instability as well as damage to the bone. Many treatment options exist. In addition to surgical interventions, conservative therapy is also possible, of course, their effectiveness depends on the type of pathology and the specific case.

Oral joint supplemets are often used meet the increased demands of micro- and macronutrients or to improve performance, prevent and/or eliminate problems. Many products with many ingredients are available on the market, but several researchers have found that there is a large number of poor quality supplements ready to be sold to innocent customers, even veterinarians. In the last years the online sales increased the availability of these products, and because of the high popularity of the equine oral joint supplements many new manufacturers appeared further increasing the range of products. Despite the easy access, it is still difficult to buy a good quality supplement which is safe and effective.

The objectives of this thesis were to evaluate the content of websites for the quality of information available to consumers and for the presence of a complete list of ingredients, to evaluate whether the labelling of products is appropriate and whether the ingredients used have a scientific evidence for use in horses.

2. Literature review

2.1 The equine joint

Joints consists of two adjacent bones connected together. They can allow for movement through muscle contractions as these result in the shortening of the muscle fibres which in turn pull the tendons that connect the muscles to the bones [1].

The equine joints can be classified as fibrous, cartilaginous or synovial [2].

Fibrous joints are called like so as the bones are linked by fibrous tissue. As horses grow, this tissue undergoes ossification, rendering these joints unmovable [2].

As the name suggests, cartilaginous joints, such as those found between the vertebrae in the back, are united by either fibrous or hyaline cartilage. Whichever type it is, the cartilage has the ability of limiting or even inhibiting the movability of the joint [2].

Synovial joints are the most mobile joints, thus, they are the ones which are most likely to get injured. The main components of this joint type are articular cartilage, synovial membranes which produce synovial fluid, fibrous joint capsules and collateral ligaments. The articular cartilage, which is generally hyaline cartilage, surrounds the bones [2]. It does not contain any blood vessels or nerves which makes the healing capacity poor [1]. The cartilage consists of a well-organised collagen network consisting mainly of type II collagen fibrils and an extracellular matrix (ECM). The ECM consists of aggrecan molecules and water. The aggrecan molecules are aggregates of proteoglycan molecules on a hyaluronic acid molecule. The proteoglycan molecules are a protein backbone with negatively charged chondroitin sulfate and keratin sulfate molecules. The repulsion of these negatively charged side chains and the attraction of the positively charged water molecules to them partly contribute to the compressive resistance of these joints [3]. This structure makes the cartilage act like a ‘sponge’; it removes and absorbs water from the matrix according to the pressure inside the joint so that it is evenly distributed [1]. **Figure 1** demonstrates the structure of the articular cartilage [4].

The fibrous joint capsule is lined by the synovial membrane, and it runs along the sides of lateral sides of the bones so that it surrounds, protects and, along with the ligaments, supports the joint. The synovial membrane produces synovial fluid which allows the bones to slide over each other without any friction [2]. Together with the synovial fluid, the synovial membrane, allows nutrients to move to and from the blood throughout the joints. The synovial fluid consists of proteins, enzymes, water, leukocytes, but the most important

component is sodium hyaluronate which is a coiled, negatively charged sugar chain, also known as a glycosaminoglycan. The latter contributes to the compressive resistance of the joint since it has a shock-absorbing capacity [1].

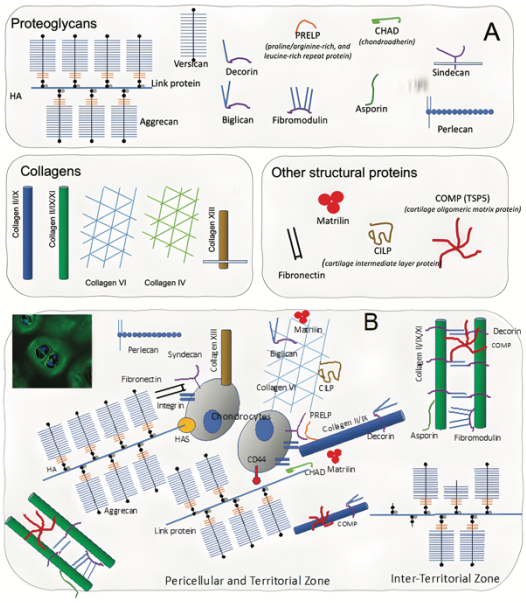

Figure 1: articular cartilage ECM: components (A) and organisation (B) [4].

2.2 Osteoarthritis

According to a survey that was carried out, the most prevalent cause of lameness is osteoarthritis (OA); it accounts for 60% of lameness problems in horses [5]. OA is a musculoskeletal condition compromised of biologic and pathologic processes which ultimately lead to the disintegration of the articular cartilage [6]. The reason behind joint damage is inflammation. Inflammation is a physiological process which occurs after an injury with the aim of decomposing and eliminating the foreign material [1]. However, in case of OA, this process becomes pathological due to the release of several harmful mediators from the inflamed synovial membrane or due to trauma. This results in the initiation of a cascade, which is mainly triggered by the cytokine interleukin (IL) -1. This cascade results in the release of metalloproteinases, aggrecanase and prostaglandin E2 (PGE2) [3]. The inflammation causes a change in the chemical composition of the synovial fluid which leads to a decrease in the fluid's viscosity, and inefficient repair processes by the chondrocytes due to improper nutrient supply. All of these pathologies damage the cartilage and expose the bone ends to trauma. The bone tries responds with a defense mechanism which only makes the situation worse and leads to the formation of osteophytes and sclerosis [1]. The high prevalence of OA and the lack of a definite cure makes oral joint

supplements (OJSs) the most common type of supplements purchased by horse owners. This is proven by the fact that a third of equine supplement sales in the USA are accounted for by OJSs. In addition to this, in a study regarding the feeding regimen of three-day-event horses, OJSs were one of the four oral supplements that owners administered to their horses daily [5].

2.3 The Equine Diet and Supplementation

Supplementation can have two different definitions which will be discussed below.

The major component of all horses' diets is forage. However, in certain cases, forage does not provide enough nutrients for maintenance, let alone for pregnant, lactating or animals with a very high activity level. Thus, supplementation is required to meet these increased levels of micro- and macronutrients. This form of supplementation is achieved through the introduction of proprietary feeds or cereal grains into the diet; these can be referred to either "by a generic term (complementary feeds or concentrates) or by the feed form (sweet feed, pelleted feed, compound feeds, etc.)" [7].

Administering a complementary food is a form of supplementation which refers to the products used as 'special supplements' since their aim is not to add any nutritional value to the diet but to improve performance, prevent any kind of musculoskeletal disorders and eliminate or control a problem [7]. There is evidence that the negative process triggered by osteoarthritis (OA) can be influenced by these supplements in vitro, since they are able to provide building blocks, however, it is uncertain whether this is also the case in vivo after these supplements have undergone digestion. Thus, additional, objective, randomised clinical trials are necessary to prove these claims [6]. The European Union does not give a legal definition for this word, and this creates room for confusion. Due to this lack of clarity, "all such products or ingredients must be labeled as complementary feedstuffs, even though in practice owners and feeders clearly differentiate between traditional complementary feeds as described above and supplements". Lack of clarity also lies within the fact that these specialised supplements may also be referred to as a 'nutraceutical'. According to the definition given by the Foundation of Innovation in Medicine (1991), "Nutraceuticals have been defined as 'any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease'". This includes nutrients and their derivatives as well as herbs. However, in several countries, it is illegal to sell feed products that claim that they can prevent or treat a disease. The word 'nutraceutical' can be broken down into two parts: 'nutra' which refers to nutrients and 'ceutical' which

refers to a medical drug [7]. However, these supplements are neither food nor medical drugs, thus, they have advantages over either of them [6].

Feed is 'generally recognised as safe', however, nutraceuticals, even though they may contain natural substances, are not necessarily safe. Due to this lack of safety information, it is prohibited to include nutraceuticals in the ingredient list. Furthermore, since they are not regarded as food, manufacturers are not required to list the ingredients nor the nutrient profile. One also needs to keep in mind that, according to a study carried out, even when the active ingredients are listed, they might not be present in the concentrations claimed on the label which may render them ineffective. Unlike drugs, nutraceuticals do not have to undergo a lengthy drug approval procedure to prove their safety and efficacy since they are not regarded as such, even though, according to the definition above, these products claim to have the same effects [1].

In the USA, manufacturers are not required to: register themselves or their products with the Food and Drug Administration, follow Good Manufacturing Practices, which are put in place to ensure that the product batches are consistently of good quality, and monitor after production [6].

Regulation (EC) number 1831/2003 of the European Parliament and of the council has the scope of establishing "a Community procedure for authorising the placing on the market and use of feed additives and to lay down rules for the supervision and labelling of feed additives and premixtures in order to provide the basis for the assurance of a high level of protection of human health, animal health and welfare, environment and users' and consumers' interests in relation to feed additives, whilst ensuring the effective functioning of the internal market" [8].

2.4 The ACCLAIM System

There is a high number of easily accessible poor-quality supplements on the market. A supplement is considered to be of poor quality when: it does not incorporate the ingredients on the label, it does not incorporate the ingredients in the amount listed on the label, the amount administered to the horse is less than that necessary to cause an effect and it is contaminated with damaging constituents or other supplements. Therefore, for these reasons, the ACCLAIM system, shown in **Figure 2**, exists. This is a system consisting of seven steps which aids in the choosing of nutritional supplements as it ensures that they are safe, effective and of good quality [5].

	Variable	Description
A	A name you recognize?	Is the product in question manufactured by a company you recognize? Products manufactured by established companies that provide educational materials for veterinarians and consumers are preferable to OJHSs manufactured by newly formed companies.
C	Clinical experience	Companies who support clinical research and have their products tested in clinical trials for safety, efficacy, and bioavailability with results published in peer-reviewed journals are more likely to have a quality product. These publications should be readily accessible to veterinary practitioners, and companies should be able to provide copies of their published research for your review. Some manufacturers claim to have their product tested but are subsequently unable to provide data or a reprint for evaluation.
C	Contents	All active and inactive ingredients and fillers should be indicated on the product label. Products that do not contain the amount of ingredients as listed on the product label likely contain other fillers or ingredients that are not identified and therefore may pose a potential health risk to the horse or the person administering the supplement.
L	Label claims	If the claims sound too good to be true, they probably are. Supplements with realistic label claims based on scientific study results, rather than testimonials, are preferable. Illegal claims such as those claiming to diagnose, treat, cure, or prevent a disease are abundant. Products with illegal claims should be avoided.
A	Administration recommendations	The amount of active ingredient administered per dose per day should be easily calculated. A product with some ingredients listed in milligrams and others in ounces but presenting the overall dosing instructions in scoops, without detailing amount of ingredients per scoop, are deliberately confusing. Look for products with clear administration recommendations with the recommended dosages based on published clinical trials.
I	Ingredients	Products with a lot identification number or some other tracking system suggest that some form of pre- and/or post-market surveillance system to ensure product quality is in place. Companies that have voluntarily instituted current Good Manufacturing Practices (cGMPs) and other quality-control/quality-assurance techniques (e.g., tamper-resistant packaging and individual tablet/caplet identification) are more likely to be reputable. Producing a supplement akin to a pharmaceutical drug shows a long-term investment into their product and company.
M	Manufacturer information	Manufacturer information should be clearly stated on the label, preferably in concert with contact information or a website for customer support. Companies employing veterinarians to answer technical questions/issues are preferred.

Figure 2: Seven-step ACCLAIM system [5]

2.5 Glucosamine

2.5.1 Forms of glucosamine

Glucosamine can be found in three forms in nutritional supplements: glucosamine hydrochloride, glucosamine sulfate and N-acetyl-D-glucosamine (NADG). Glucosamine hydrochloride and sulfate are more effective at preventing the degeneration of cartilage than NADG. “glucosamine HCl is the most stable form and is twice as bioavailable as the alternatives. In controlled blind studies, those with positive outcomes used the HCl form” [9]. Jantzen (2008) supported this statement made by Anderson and Rendle (2015) by stating that a higher percentage of free glucosamine (approximately 80%) can be obtained from glucosamine hydrochloride than glucosamine sulfate (approximately 50-60%) [2]. However, Block et al. (2010) contradicted this statement in two ways: first, by saying that there is a common misconception among the general public that glucosamine sulfate is better than glucosamine hydrochloride and second, by saying that, in reality, neither of the two glucosamine salts are superior [10]. Another study supports this statement made by Block et al. (2010) as it was concluded that the bioavailability of the nutritional supplement

administered orally is not affected by the salt [11]. This study made by Block et al. (2010) is contradicted by a study in which it was concluded that the average bioavailability of glucosamine sulfate was 9.4 % while that of glucosamine hydrochloride was 6.1% [12].

2.5.2 Mode of action

Glucosamine is the building block of cartilage glycosaminoglycans (GAGs). Furthermore, due to its ability to decrease the levels of prostaglandin E2 and the expression of cyclooxygenase (COX) -2, it has an anti-inflammatory effect [9].

2.5.3 Efficacy

In one study, radioactive glucosamine was shown to arrive and distribute itself into the cartilage. However, in another study study, it was mentioned that since glucosamine sulfate is a large molecule its incorporation into the cartilage is not possible [11].

Unfortunately, only a small number of studies were done *in vivo* to see whether the effects of glucosamine observed *in vitro* also occur *in vivo* and the results are inconsistent [6]. In fact, assuming that the results observed *in vitro* will be seen *in vivo* when giving the same doses is not correct since according to data from two studies mentioned by Lamprecht (2010), the concentrations of the nutritional supplements reaching the blood and synovial fluid *in vivo* are significantly lower than those in *in vitro* models of osteoarthritis. These studies are also relevant for chondroitin sulfate (CS) [13].

At the time of my research work, no clinical studies investigating the individual effects of glucosamine have been carried out in horses.

2.5.4 Dosage

The minimum plasma concentration at which glucosamine is effective in both humans and animals is still unknown [11]. Increased levels of glucosamine in the synovial fluid of horses were observed after administration of 20 mg/kg of glucosamine via a nasogastric tube (NG tube) [12]. In one study it was concluded that 15 grams of glucosamine per day is sufficient while in another study recommended 20 mg per kg body weight which would amount to 12 grams of glucosamine per day in a 600 kg horse [5].

2.6 Chondroitin

CS, obtained from bovine and shark cartilage, along with glucosamine, is one of the most common ingredients found in equine joint supplements [6]. How effectively chondroitin is absorbed due to its large size is a question up for debate [1]. In a study mentioned in a report, 3 grams of chondroitin were administered via a nasogastric tube, and it was shown to have a bioavailability of 22% [2].

2.6.1 Mode of action

CS as well as glucosamine are responsible for slowing down the breakdown of cartilage through the blocking of degrading enzymes e.g. collagenase and intermediate mediators e.gs. PGE2, nitric oxide and nuclear factor kappa B (NF-κB). Furthermore, they are responsible for the synthesis of the extracellular matrix as they provide the building blocks required for this, they increase gene expression as well as activate cell receptors and cell signalling mechanisms. CS has anti-inflammatory properties which are due to its ability to block chemotaxis as well as its ability to decrease phagocytosis and lysozyme release amongst other reasons [2].

2.6.2 Efficacy

Similar to glucosamine, *in vivo* studies concerning chondroitin are lacking. A study involving mares with induced osteoarthritis lesions supplemented with CS (2.5 g/day) found decreased levels of PGE2 in the synovial fluid, production and distribution of matrix metalloproteinase-3 as well as GAGs. This study also found that this supplementation bettered "maximum flexion angle as well as a decrease in joint circumference" [13].

A study concluded that the likelihood of reaching effective concentration of CS in the blood and synovial fluid increases if several repeated doses are administered orally as it allows the active ingredient to accumulate over a period of time [13].

2.6.3 Dosage

A study reported that 2-6 grams of chondroitin per day has several beneficial effects as it provided relief from certain symptoms as well as led to the improvement of stride characteristics and length, the joint motion and duration of the swing [5].

2.7 Combination of glucosamine and chondroitin

Often times, glucosamine and CS are found together in the same product. One of the reasons for this might be the expensive price of chondroitin, thus, manufacturing a pure form of it would result in a pricy product [9]. Another reason is that multiple studies have shown that combining glucosamine and CSU has a positive outcome [14]. Glucosamine is responsible for increasing the production of GAGs while chondroitin prevents the matrix degeneration [2].

2.7.1 Efficacy

In a study, 16 horses' metacarpophalangeal joint were investigated. 8 of the horses were treated with a combination of glucosamine and CS while the other 8 served as a control group. The results of the study confirmed the beneficial effects of this combination, with the horses in the treated group having lower lameness scores, lower levels of PGE2 which were proven by the lower level of inflammation on the ultrasound and less frequent findings on the ultrasound when compared to the horses in the control group. The authors also mentioned that this combination is only useful for controlling and preventing the effects of the disease but not for the repairment of the joint cartilage or decreasing the catabolic processes in joints with a substantial amount of damage [14].

2.7.2 Dosage

A study concluded that 15 grams of glucosamine and 2 grams of CS improve the joint motion range and stride length after 8 weeks of treatment and the swing duration after 12 weeks of treatment [2].

2.8 Green lipped mussel (*Perna Canaliculus*) extract

The first oral joint supplement for horses contained nutrients extracted from the green lipped mussel [15]. These nutrients include essential FAs, CS and glucosamine. Therefore, this mussel's extract possesses the potential beneficial effects of CS and glucosamine as well as an effect on the enzymes involved in joint inflammation as a result of the presence of the essential FAs [9].

The green lipped mussel has pernin, a "non-pigmented, aggregating and glycosylated protein". This protein is found in a high concentration in the haemolymph. Pernin hinders the activity of serine protease and since it is the only active protein in the extract, it is thought

to be responsible for the beneficial properties that the mussel possesses [15]. However, Eason et al. (2018), stated that the following are bioactive ingredients present in the extract of the mussels: fatty acid fraction, carotenoids, pro-resolving lipid mediators, sulphated polysaccharides, and F-acids. The fatty acid fraction is known to suppress the action of 5-lipooxygenase (5-LOX) which leads to a decrease in the harmful activity of leukotrienes in arthritis. The rest of bioactive ingredients have potential anti-inflammatory effect [16].

2.8.1 Efficacy

A randomised, placebo-controlled, double blinded study was done to investigate the effects of green-lipped mussel in horses with chronic fetlock lameness as a result of OA. The results demonstrated that a dose of 25 mg/kg "significantly alleviated the severity of lameness, joint pain and improved response to joint flexion in affected fetlock joints as assessed by veterinary lameness examinations" [17].

In a trial involving 10 horses with IL-1 induced inflammation, the horses supplemented with green lipped mussel extract along with other ingredients, two of them being shark cartilage and abalone, had no increase in synovial fluid PGE-2 and sulphated GAGs as opposed to the control horses receiving no supplementation. Moreover, the horses in the control group also had increased synovial fluid protein and leukocyte levels. However, it is not clear which active substance is responsible for the effects observed [16].

However, the efficacy of this mussel is variable. This might be due to the fact that even though the studies use different methods of preparation for the extract, they still all refer to the preparate as 'green-lipped mussel extract' [15].

2.9 Shark cartilage

In a study mentioned under 'Green lipped mussel (*Perna canaliculus* extract)', a supplement containing multiple active ingredients including shark cartilage was investigated [16]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of shark cartilage have been carried out in horses.

2.10 Avocado soybean unsaponifiables (ASU)

As the name suggests, this product is produced by obtaining the unsaponifiable oil extracts obtained from avocado and soybeans and then mixing them in different ratios [6].

2.10.1 Efficacy

A study which was carried out for 70 days involved horses being divided into two groups: one group received the ASU supplement along with molasses while the other group acted as a control group and received only molasses. There was no significant difference in the pain and lameness of the horses receiving the supplement when compared to those not receiving it. However, there was a significant decrease in the synovial membrane inflammation and in the cartilage disease score of the horses receiving the supplement when compared to those that did not. Due to this decrease in the cartilage disease score, ASU can be regarded as a disease-modifying osteoarthritic drug [6].

2.11 Cetyl myristoleate (CM)

Cetyl myristoleate is a "fatty acid that is being used in equine joint supplements. CM is an ester of cis-9-tetradecenoic acid (myristoleic acid) and 1-hexadecanol (cetyl alcohol) and is a 14-carbon monounsaturated omega-5 fatty acid" [6].

A study which aimed at investigating the effects of Myristol™, a product containing CM along with glucosamine hydrochloride, MSM and hydrolysed collagen (combination of active substances) found that there was significant improvement in the treatment group [6]. However, it is not clear which active substance is responsible for it Therefore, at the time of my research work, no clinical studies investigating the individual effects of CM have been carried out in horses.

2.12 Vitamin C

Vitamin C is an additive frequently found in supplements which possesses antioxidant properties [7]. Thus, it is possible that it might act against arthritis by protecting chondrocytes from oxidative damage [9]. Furthermore, it is necessary for collagen formation [1].

2.12.1 Efficacy

An *in vivo* randomised, controlled study was done to see whether vitamin C has anti-oxidative capacities in horses which were divided into two groups. The first group which received 50 g of rosehip powder which is equivalent to 250 mg of vitamin C had a significant increase in their serum vitamin C levels after 14 days and this value doubled by day 84 and a significant decrease in oxidative stress within the first 14 days. The second group which

received 25 g of rosehip powder which is equivalent to 125 mg of vitamin C and 25 g of placebo powder did not have these significant effects [18].

2.13 Vitamin E

This is a lipid soluble vitamin and it is the most frequently supplemented antioxidant in horses. Vitamin E aids in the proper functioning of the muscles, nerves and immune system. Furthermore, it is involved in keeping the structural integrity of the cell membrane [7]. At the time of my research work, no clinical studies have been carried out in horses.

2.14 Fenugreek (*Trigonella foenum-graecum*) seed

Fenugreek has anti-inflammatory, antioxidant and analgesic effects manifested through its active ingredients' ability to suppress the activity of the lipid peroxidase, COX-1 and COX-2 enzymes. The latter two enzymes are involved in the transformation of arachidonic acid (ARA) to prostaglandins (PG) [19].

2.14.1 Efficacy

A study was done to investigate the effect of DigestaWell NRG®, a supplement synthesised from yucca (*Yucca shidigera*) and fenugreek on the concentrations of the circulating inflammatory cytokines. The study included 20 horses which were divided into two groups: a treated group, supplemented with 200 grams of DigestaWell NRG® once daily for 4 weeks and a placebo group receiving no supplementation. The horses underwent a ridden standardised exercise test before and after the study. The results suggested that fenugreek, along with yucca, have anti-inflammatory properties as there was a decrease in the average concentration of TNF-α in the horses in the treated group [19]. However, it is not clear which active substance is responsible for these properties. Therefore, at the time of my research work, no clinical studies investigating the individual effects of fenugreek have been carried out in horses.

2.15 Yucca (*Yucca shidigera*)

Yucca is an herb commonly found in equine oral joint supplements along with other ingredients [7]. It has a number of active ingredients, primarily saponins [20]. These saponins are mainly steroid-like and have been shown to have anti-inflammatory, antioxidant, and antispasmodic properties [7]. Due to these properties, yucca may aid in the

alleviation the pain and discomfort which occur as a result of arthritis, bone and joints problems and/or soft tissue swelling [20]. Yucca also contains polyphenols such as resveratrol and yuccaols A-E, however these are not found in the extract but only in the bark [7].

2.15.1 Efficacy

A study mentioned under 'Fenugreek (*Trigonella foenum-graecum*) seed' was done investigating the efficacy of yucca and fenugreek and found that they possess anti-inflammatory properties [19]. However, it is not clear which active substance is responsible for these properties. Therefore, at the time of my research work, no clinical studies investigating the individual effects of fenugreek have been carried out in horses.

2.15.2 Dosage

The dosing is not known [7].

2.16 Resveratrol

This is a polyphenol having anti-oxidative, anti-apoptotic and anti-inflammatory properties. The anti-inflammatory properties are a result of the: inhibition of NF-κB which in turn prohibits the activity and production of IL-1β, scavenging of reactive oxygen species and the downregulation of pathways controlled by COX-2 [21].

2.16.1 Efficacy

A study was conducted to determine the effect of resveratrol in performance horses with lameness in the distal tarsal joints. The study consisted of 45 horses which were randomly assigned to a treatment group (23 horses) and a placebo group (22 horses). The horses in both groups were injected with triamcinolone acetonide in the centrodistal and tarsometatarsal joints of both hindlimbs. Following this, they were administered with the test articles twice daily for four months. These test articles were identical except for the fact that the test article administered to the horses in the treatment group contained 1000 mg of microencapsulated resveratrol[b]. The results suggested that the horses in the treatment group had a reduced level of lameness when compared to the horses in the placebo group. In fact, the number of horses reported to have a better performance, compared to the same or worse, was significantly higher than that of the placebo group. Furthermore, the A1:A2 ratio of the

horses in the treatment group was significantly better than that of the horses in the placebo group. The A1:A2 ratio is "the measure of vertical pelvic movement versus expected pelvic movement for each stride, and the MAXDIFF and MINDIFF values, which are measures of the degree of asymmetry of pelvis movement over all collected strides" [21].

Another study was conducted to determine the effect of resveratrol on certain oxidative biomarkers in aging and lame horses. The study consisted of a treatment group and a placebo group, each compromised of 8 horses, totalling to 16 horses all with grade 3 lameness. The horses in the treatment group were "fed four scoops (30 g) of Equithrive Joint powder containing 2,000 mg resveratrol and 200 mg of sodium hyaluronic acid and the carrier *Saccharomyces cerevisiae*" for the first 10 days of the study followed by 2 scoops (15 g) as a "maintenance dose for the remaining 18 days". The horses in the placebo group followed this same protocol however they received *Saccharomyces cerevisiae* instead of Equithrive joint powder. The following results were recorded: an increase in superoxide dismutase (SOD) and catalase enzyme activities, both of which are antioxidants. However, as opposed to the results of other studies referenced by the authors of the research paper, there was a decrease in glutathione peroxidase (GPx), another antioxidant enzyme. Finally, there was a decrease in the concentration of MDA, a marker of oxidative stress. These results suggest that Equithrive Joint powder can potentially protect against oxidative stress and aging in horses [22]. Furthermore, the following results were recorded in the treated group when compared to the placebo group: a significant decrease in the tumour necrosis factor-alpha (TNF-α), a factor associated to inflammation and a significant decrease in the erythrocyte sedimentation rate, which is used to indirectly measure the inflammation in the body. Therefore, these results suggested to the authors that Equithrive joint powder has anti-inflammatory properties as it is capable of reducing the levels of inflammatory mediators [23].

Another study was carried out on 12 horses to determine the effect of oral supplementation of resveratrol (Resvantage Equine®) on phagocytosis, oxidative burst and leukocyte production of tumour necrosis factor and IL-1 beta (IL-1β). In this study it was concluded that administering resveratrol orally for 3 weeks at the clinically recommended dose (450 mg twice daily) had no significant difference on the phagocytosis, oxidative burst and leukocyte production of tumour necrosis factor and IL-1β of the horses in the treated group when compared to the horses in the control group. The authors of the study suggested that

these contradictory results when compared to other studies might be due to the fact that recommended dose was insufficient [24].

2.17 SOD

This is an enzyme with antioxidant properties. As the name suggests, it dismutates superoxide ions into oxygen and hydrogen peroxide [25].

2.17.1 Efficacy

Studies have shown that orally supplemented SOD is bioavailable and is able to decrease the inflammatory response markers as well as oxidative stress. However, these same beneficial effects have not been observed in horses [26].

A study was carried out to reveal the effects of oral SOD supplementation in horses following powerful, tiring exercise. 12 horses were divided into two groups, a treatment group and a control group. The treatment group was supplemented with 3 grams of a SOD oral proprietary formula. The control group received 3 grams of microcrystallised cellulose powder. Both of these supplementations were added to the morning feed. The results of this study suggest that oral supplementation of SOD has no effect on the systemic markers of inflammatory response, joint health and antioxidant levels [25].

2.17.2. Dosage

The dosing is not known [7].

2.18 Garlic (*Allium sativum*)

Garlic has several active constituents one of which being allicin [20]. At the time of my research work, no clinical studies have been carried out in horses.

2.19 Methylsulfonylmethane (MSM)

MSM is an organic source of sulfur, an element important for the muscles, joints, cartilage and bones [2]. MSM has antioxidant properties and anti-inflammatory properties. Thus, due to the latter properties, it is commonly added to products that target arthritis [15].

2.19.1 Efficacy

A descriptive study was carried out in horses during a jumping competition. The horses were divided into 3 groups: a control group (no supplementation), a group receiving 8 mg/kg of MSM daily and a group receiving a combination of 8 mg/kg of MSM and 5 mg/kg of vitamin C daily. By the end of the study, the results showed that MSM is effective. Carbon monoxide levels and lipid hyperoxide levels increase after exercise; these levels still increased in the horses receiving MSM supplementation however to a smaller degree. There was also an antioxidative effect which was greater in the group of horses receiving a combination of the compounds which indicates that there might be a synergistic effect between them. Thus, from this study one may conclude that MSM might have a protective effect in exercise related inflammation and oxidative damage. Therefore, there is proof that implies that MSM supplementation to horses with inflammation or oxidative stress caused by exercise can lead to a protective effect in horses [15].

In another study involving the oral administration of a supplement containing glucosamine, CS and MSM to geriatric horses, there was no significant improvement in the gait characteristics [27].

2.19.2 Dosage

These antioxidant and anti-inflammatory properties of MSM can be achieved by supplementing 4.8 grams of MSM to horses [2].

2.20 Ginger (*Zingiber officinale*)

Ginger possesses anti-inflammatory properties which are manifested through the hindering of COX and lipoxygenase (LOX) and the decrease in the production of the tumour necrosis factor and inflammatory PG [28].

2.20.1 Efficacy

A study proved that a natural ingredient found in ginger, [8]-paradol, exerts anti-inflammatory effects on human whole blood. This ingredient inhibited COX-1 and prevented the platelets from aggregating, making ginger a possible cure for musculoskeletal disorders. However, a crossover study mentioned in this same review carried out in nine horses aimed at investigating the effects of a single dose of ginger, showed that ginger led to a rise in pro-inflammatory cytokines TNF-α and interferon-gamma (IFN-γ) [29].

In a study involving horses, results showed that ginger has no effect on the markers on oxidative stress and antioxidant status [7].

2.20.2 Dosage

Due to the lack of studies supporting the efficacy of ginger in horses, there is no recommended dose [7].

2.21 Creatine

Creatine is advertised as a performance enhancing substance for both humans and horses and it forms an integral part of creatine phosphate. It is a derivative of naturally occurring amino acids in the diet of carnivorous animals. On the other hand, in horses, creatine is most probably synthesised from arginine, L-methionine and glycine [7].

2.21.1 Mode of action

Reviews have shown that the administration of creatine can exert its beneficial effects through: the elevation of muscle glycogen and phosphocreatine (PCr) levels, the aiding in the more rapid resynthesis of PCr, the elevation of the endocrine and growth factors mRNA expression or the indirect work when the amount of work increases [7].

2.21.2 Efficacy

A study which involved the feeding of horses with 50 mg of creatinine per kg bodyweight per day suggested that creatine has a poor absorption from the intestinal tract of horses. This is because even though there was a twofold increase in the plasma creatine concentration, there was no increase in the creatine levels in the muscles [7].

A study involving the supplementation of “100-120 mg/kg bodyweight creatine monohydrate per day for 14 days” also showed that there was no increase in the creatine levels in the horse muscles as well as “no effect on performance parameters or muscle metabolic responses to exercise” [7].

Therefore, there are currently no studies supporting the use of creatine as a performance enhancing substance in horses [7].

2.21.3 Dosage

The effective dose is unknown [7].

2.22 (alpha-)Lipoic acid

Lipoic acid (LA) is “an eight-carbon structure that contains a disulfide bond as a part of a dithiolane ring with a five-carbon tail” [7]. LA and its reduced form, dihydrolipoic acid, can regenerate antioxidants such as glutathione, ascorbic acid and alpha-tocopherol [27]. It is known for its antioxidant properties which are thought to have a preventative and therapeutic effects in humans and laboratory animals. Lipoic acid is different to other antioxidants as it is soluble in both water and fat, thus, it can act it in the cell membrane and the cytoplasm [7].

2.22.1 Efficacy

In a study, horses in the groups supplemented with α-LA and vitamin E had a: 40% higher level of glutathione, 30% higher level of plasma α-tocopherol and a 15% higher level of plasma ascorbic acid when to the horses in the control group. These results prove the radical scavenging and recycling ability of supplemented vitamin E and α-LA [27]. However, it is not known which of the ingredients is responsible for this effect. Therefore, at the time of my research work, no clinical studies have been carried out in horses.

2.22.2 Side effects and dosage

No adverse effects have been recorded for the dose of 10 mg/kg bodyweight per day used in research studies. Furthermore, this high dose is never used in supplements as it would lead to the product being too bitter and expensive [7].

2.23 Devils claw (*Harpagophytum procumbens*)

Devils claw is a herb containing the following active ingredients: iridoid glycosides, acetylated phenolic glycosides and terpenoids [7]. It is on the prohibited substance list of the FEI [30].

Devils claw is marketed for its anti-inflammatory and analgesic properties and certain human studies support these claims as the results recorded a “decrease in pain intensity and an increase in flexibility”. These results could be conveyed to equine medicine [7]. Devils claw most common indication in equine medicine is to provide relief from pain originating from degenerative joint disease [20].

2.23.1 Efficacy

A study showed that orally supplemented devil's claw is effective at treating inflammation, arthritis, rheumatoid arthritis (RA) and tendonitis [20].

In a blind, cross-over designed study, six horses with osteoarthritis were used to investigate the effects of a joint supplement containing devil's claw, burdock (*Arctium lappa*), nettle (*Urtica dioica*), dandelion (*Taraxacum officinale*) and comfrey (*Symphytum officinale*). The results showed a decrease in the PGE2 levels in the synovial fluid which prove the herbs' anti-inflammatory effect. However, the majority of the horses did not have a better lameness score at the end of the study when compared to the beginning [31]. However, it is not clear which active substance is responsible for these anti-inflammatory properties.

The results of another study investigating a similar blend of herbs as the one mentioned, also demonstrated their anti-inflammatory properties on an OA cartilage model [31]. However, it is not clear which active substance is responsible for these anti-inflammatory properties.

Another study comparing the effects of an herbal blend consisting of devil's claw, black currant (*Ribes nigrum*), horsetail (*Equisetum arvense*) and white willow (*Salix alba*) to those of phenylbutazone found that, the horses supplemented with the herbal blend had a lower average lameness score (6) than those that were administered phenylbutazone (8.6) [31]. However, it is not clear which active substance is responsible for these anti-inflammatory properties.

Therefore, at the time of my research work, no clinical studies investigating the individual effects of devils claw have been carried out in horses.

2.23.2. Dosage

The effective dose of devils claw is unknown [7].

2.24 Burdock (*Arctium lappa*)

Studies mentioned under 'Devils claw (*Harpagophytum procumbens*)' have investigated the effects of a herbal blend with one of the constituents being burdock [31]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of burdock have been carried out in horses.

2.25 Nettle (*Urtica dioica*)

Studies mentioned under 'Devils claw (*Harpagophytum procumbens*)' have investigated the effects of a herbal blend with one of the constituents being nettle [31]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of nettle have been carried out in horses.

2.26 Dandelion (*Taraxacum officinale*)

This is a worldwide growing weed whose leaves and roots can be used for several reasons.
Studies mentioned under 'Devils claw (*Harpagophytum procumbens*)' have investigated the effects of a herbal blend with one of the constituents being dandelion [31]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of dandelion have been carried out in horses.

2.27 Comfrey (*Symphytum officinale*)

This is a worldwide growing weed whose leaves and roots can be used for several reasons.
Studies mentioned under 'Devils claw (*Harpagophytum procumbens*)' have investigated the effects of a herbal blend with one of the constituents being dandelion [31]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of comfrey have been carried out in horses.

2.28 White willow (*Salix alba*)

This is a plant which similarly to meadowsweet contains salicylate and thus, may lead to a positive result when testing for drugs [31].
A study mentioned under 'Devils claw (*Harpagophytum procumbens*)' have investigated the effects of a herbal blend with one of the constituents being white willow [31]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of white willow have been carried out in horses.

2.29 Turmeric (*Curcuma longa*)

Curcumin is a substance extracted from turmeric known for its anti-inflammatory effects which it exerts through the inhibition of COX-2, PG and leukotoxins [28]. It also possesses antioxidant properties. Thus, it could probably be used for the treatment of inflammatory conditions such as OA [32].

Curcumin's "bioavailability is poor because of poor absorption, poor solubility in aqueous solution, rapid metabolism and rapid systemic elimination". In ruminants, curcumin is fermented into short chain fatty acids (FAs) by the microbes in the rumen which leads to the slowing down of excretion. It is thought that the hindgut of horses acts in a similar way, however, further research is necessary [33]. Still, care must be taken when evaluating *in vitro* studies for efficacy as the concentrations used in them may not be achievable *in vivo* [32].

2.29.1 Efficacy

Two trials were done aimed at investigating the effect of a proprietary formula containing curcumin and vitamins E and C. The first trial included the addition of 30 grams for 30 days of proprietary formula once daily to the normal feed of two horses. The second trial was comprised of six horses having different levels of osteoarthritis. All the horses' lameness scores improved by at least one grade, most of them by two grades, by the end of the 30 days. Furthermore, one horse in the second trial that had synovial effusion at the start, showed a 70% improvement at the end of the trial [34]. However, it is not clear which active substance is responsible for the effects observed.

A pilot study involved 12 horses different into two groups: the first group was a control group receiving no curcumin supplementation while the second group was supplemented with 15 grams of 95% curcumin daily for 30 days. The erythrocyte sedimentation rate was evaluated from blood samples taken before and after a ride on several days to investigate the effects of the supplementation on inflammation. The results suggest that curcumin needs to be supplemented for a minimum of 14 days to exert its anti-inflammatory effect. Still, there was no significant difference in the level of inflammation at the end of the study when compared to the start [35].

2.29.2 Dosage

The daily dosage varies, it can be anywhere between 180-2000 mg. For this reason, it is difficult to compare studies [28].

2.30 Aloe vera

The aloe vera plant's extract or gel contains a high number (75) of possible active ingredients which have the following effects: antioxidant, anti-inflammatory and cytoprotective [20]. Due to these claimed anti-inflammatory effects, manufacturers of joint health supplements use aloe vera in combination with omega-3 FAs to act against arthritis in animals [36].

2.30.1 Efficacy

Several studies which prove aloe vera's ability to fight inflammation, however, none of them were carried out on horses. The following results were obtained from these studies:a decrease in prostaglandin production due to a decline in arachidonic acid (ARA) oxidation, a 30 % and 20 % suppression in the synthesis of PGE-2 and IL-8 respectively, a decline in TNF-α and IL-6 levels and a 50 % and 48 % decrease in synovial pouch swelling and mast cell production respectively [37].

A study comparing 2-, 3- and 4-year-old aloe vera plants, results demonstrated that 3-year-old plants are the superior radical scavengers as they have the most flavonoids and polysaccharides [37].

Therefore, at the time of my research work, no clinical studies have been carried out in horses.

2.30.2 Dosage

The recommended dose for a horse weighing approximately 500 kg is 200-250 ml [36].

2.31 Boswellia (*Boswellia serrata*)

Boswellia serrata's gum resin contains boswellic acids (BAs). Studies suggest that there are six main BAs, namely "keto-β-boswellic acid (KBA; a), 3-O-acetyl-11-keto-β-boswellic acid (AKBA; b), α-boswellic acid (α-BA; c), β-boswellic acid (β-BA; d), 3-O-acetyl-α-boswellic acid (α-ABA; e), and 3-O-acetyl-β-boswellic acid (β-ABA; f)". All of these can act against inflammation. These BAs have two isomers: α and β, with the β-isomer being more effective than the α-isomers. Out of all of them, AKBA is the most efficient as it is the best at suppressing the 5-LOX pathway, followed by KBA. AKBA and KBA are able to suppress the NF-κB responsible for controlling the proinflammatory cytokines' (such as TNF-α and IL-1β) cascade. "The Structure-Activity Relationship (SAR) studies on the BAs suggested that the carboxylic group and the 11-keto-group were essential for 5-lipoxygenase

inhibition, and the acetyl-group on position C-3 OH had a moderate influence on the enzyme inhibition" [38]. As a result of the TNF-α suppression, the expression of matrix metalloproteinase (MMP) -3, -10 and -12 is inhibited [39]. At the time of my research work, no clinical studies have been carried out in horses.

2.32 Liquorice (*Glycyrrhiza glabra*)

The liquorice root contains several triterpenoid saponins, the main one being glycyrrhizin. The ameliorative potential of glycyrrhizin is due to aglycone (18β-glycyrrhetinic acid) which has a similar structure to steroids [40]. At the time of my research work, no clinical studies have been carried out in horses.

2.33 Omega-3 fatty acids

Omega-3 fatty acids (FAs) are essential which means that they are obtained from the diet. "The 'parent' fatty acid" is "alpha-linoleic acid (ALA) for the n-3 family". There is evidence to suggest that omega-3 FAs, namely ALA and the compounds derived from it, mainly eicosapentaenoic acid (EPA) but also docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), have an anti-inflammatory effect in several species [41].

ALA is present in forage, the main constituent of any horse's diet, thus, most horses reach the body's requirements without the need for supplementation. However, horses under training need to be supplemented with grains or oils to meet their increased energy demands "Plant oils such as linseed, soybean and flaxseed oils are sources of ALA". As already mentioned, EPA, DPA and DHA can be produced from ALA. However, studies have proved that ALA has a poor conversion rate. In fact, two studies demonstrated that supplementing horses with ALA can elevate the concentration of circulating EPA but not that of DHA. Thus, a study suggested providing EPA and DHA directly, for example through the supplementation of marine fish oil. Fish oil can provide these active ingredients as fish consume algae which synthesise omega 3 polyunsaturated FAs, EPA and DHA [41].

2.33.1 Mode of action

A study showed that supplementation with EPA and DHA decreases the level of ARA in the phospholipids found in the cell membrane. Furthermore, it was demonstrated that elevated levels of EPA can prevent the metabolism of ARA and reduce pro-inflammatory COX-2 gene expression [41].

2.33.2 Efficacy

A study was done to investigate the effects omega-3 supplements on the amount of FAs in the plasma, red blood cells and skeletal muscles and thus, determine the best source. The study consisted of three groups of horses supplemented with a marine-derived (algae and fish oil), flaxseed-derived supplement and nothing (control) respectively. The number of FAs in the plasma and red blood cells only increased in the horses being supplemented with the marine-derived supplement, however the levels recorded were still below the baseline level. On the other hand, the number of FAs in the skeletal muscles increased in all three groups, with the highest level recorded in the horses supplemented with a flaxseed-derived supplement [41].

A study was conducted to investigate the effects of omega-3 long chain polyunsaturated FAs (ώ-3LCPUFA) on the amount of PGE2 in synovial fluid, the PGE2 concentration of the horses receiving a supplement with marine-derived omega-3 was lower than that of the horses that received a supplemented with flaxseed-derived omega 3 or no supplementation. Still, all the horses had a PGE2 concentration which falls within the normal range. The authors suggested that this lack of significant difference in the PGE2 concentration between the different groups might be due to the fact that the mares used for the study had no history of joint problems and/or lameness. The results of this study also suggest that ώ-3LCPUFA can reach the joints, as, the amount of LA in the supplemented horses was higher than in the non-supplemented horses. When comparing flaxseed-derived omega -3 and marine derived omega -3, the horses supplemented with the latter had a higher concentration of EPA and DHA [42].

2.33.3 Side effects

Omega-3 FAs are used as substitutes for NSAIDs (non-steroidal anti-inflammatory drugs) and COX-2 drugs in the treatment of OA which is good since the horses will experience less side effects in the long run [43].

2.34 Cod liver oil

This is a fish oil which contains the omega-3 FAs derivates EPA and DHA [44]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.35 Omega-6 fatty acids

Similar to omega-3 FAs, omega-6 FAs are also essential FAs. The 'parent' fatty acid is linoleic acid (LA). LA can be transformed into ARA which in turn can synthesise eicosanoids. There are several classes of eicosanoids however the most common ones are prostaglandins, thromboxanes, leukotrienes and lipoxins. PGE2 from the prostaglandin family plays a role in the inflammatory response and subsequently OA. In fact, it is the main target of drugs and oral joint supplements. Sources of omega-6 FAs include grains such as corn and barley as well as corn oil [41].

Omega-3 and omega-6 as well as their ratio are necessary for physiological immune responses. This is because whilst an optimal ratio supports healthy immune responses, extremely non-optimal ratios can promote immunosuppression or chronic inflammatory states [13]. At the time of my research work, no clinical studies have been carried out in horses.

2.36 Hyaluronic acid (HA)

HA is a non-sulfated glycosaminoglycan produced by type B synoviocytes and hyaluronan synthase. It is the leading constituent of ECM but it is also found in the bone marrow as well as the joint cartilage and fluid. Due to its high viscosity and "excellent viscoelasticity, high moisture retention capacity, high biocompatibility, and hygroscopic properties" HA "acts as a lubricant, shock absorber, joint structure stabilizer". From this, one can conclude that HA is involved in maintaining the physiological structure and function of the joints [45].

2.36.1 Efficacy

There are several studies investigating the effects of HA injected intravenously or intraarticularly which prove its efficacy, however, the same cannot be said for orally supplemented HA. Thus, information regarding the efficacy and bioavailability of oral HA is limited [15].

A study was done to investigate the effects of Equithrive joint supplement (30 grams loading dose and 15 grams maintenance dose), containing resveratrol and hyaluronic acid (6.7 mg/g) on lame horses due to arthritis. The results showed that this supplement decreased the level of CK, an enzyme which increases in the blood in case of muscular problems and lameness. Furthermore, this decrease proposes that the ingredients in the supplement have antioxidative capacity. However, it is not clear which active substance is responsible for the

effects observed [46]. A study was done to investigate the effects of 100 mg of hyaluronan gel administered orally after surgery for 30 days on tarsocrural joints having effusion and fragments as a result of osteochondritis dissecans. The results led to a significant decrease in the effusion compared to a control group. Despite this, another study did not demonstrate this significant difference [9].

2.37 Celery (*Apium graveolens*) seeds

2.37.1 Efficacy

A randomised, controlled study was carried out for 59 days to investigate the effects of Indian celery seeds' extract on horses with OA. A dose of 30 grams led to the manifestation of celery's analgesic properties which, in turn, led to trainers reporting that the horses' had better mobility and tolerance to exercise carried out for an extended period of time [47].

2.37.2 Dosage

A dose of 30 grams is necessary for the manifestation of celery's beneficial effects [47].

2.38 Vitamin K3

There are two naturally occurring forms of vitamin K called phylloquinone (PK), also known as vitamin K_1, and menaquinone (MK-n), also known as vitamin K_2. There is also an artificial form called menadione (MD), otherwise known as vitamin K_3 [48].

Vitamin K, being a fat-soluble vitamin is absorbed better in the presence of dietary fat. However, unlike other fat-soluble vitamins, vitamin K needs to be regularly consumed as it is not stored for a long period of time. MK-n can have several side chains which vary in length and this variation affects the absorption efficacy. It is thought that vitamin K is transported to bones via chylomicrons, mainly low-density lipoproteins [48]. Vitamin K intake was found to be inversely proportional to the occurrence of OA [49].

This vitamin plays an important part in the action of gamma-glutamyl carboxylase. This enzyme plays a role in the gamma-carboxylation of several proteins, including matrix Gla protein and Gla-rich protein. These carboxylated proteins are thought to exert a cartilage protective effect by preventing the calcification of articular cartilage [49].

Vitamin K also acts a ligand for the steroid and xenobiotic receptor (SXR) and pregnane X receptor. Transcriptional regulation via these two receptors may also be another explanation of how vitamin K protects the cartilage [49].

At the time of my research work, no clinical studies have been carried out in horses.

2.38.1 Dosage

A study suggested that 7 mg of vitamin K daily to horses without access to a fresh green pasture results in the carboxylation of 90% of osteocalcin (a vitamin K dependent protein) [48].

2.39 Bromelain

Bromelain is an enzyme extract obtained from pineapple stems. it can withstand a wide pH range, thus, protection of the protease content from the acidic pH of the stomach is probably not necessary. However, it might be necessary to protect the enzymes from digestion by acid proteases found in the alimentary tract. For better absorption, bromelain ca be given with a buffering agent such as bicarbonate or in water [50].

Bromelain's anti-inflammatory properties have been demonstrated *in vitro* and in mice [50]. It also possesses anti-oxidative properties [51]. At the time of my research work, no clinical studies have been carried out in horses.

2.40 Chamomile (*Matricaria chamomilla*)

The flavonoid content in the chamomile flowers is responsible for this herb's beneficial effects [52]. At the time of my research work, no clinical studies have been carried out in horses.

2.41 Collagen

In a randomised, controlled study, Smarkpak Equine LLC was evaluated for its effects on joint health [53]. It is a product "containing glucosamine, chondroitin sulfate, hyaluronic acid, MSM, turmeric, resveratrol, collagen, silica, and boron". Horses were supplemented with 100 grams daily. The results demonstrated that the product did not have an effect on type II cartilage synthesis or breakdown but there was an increase in the average serum collagenase cleavage neopeptide (C2C) and carboxypeptide of type II collagen (CPII). Increased levels of CPII, also known as chondrocalcin, in the serum indicate that the cartilage has undergone reparation processes in recent times [53]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work,

no clinical studies investigating the individual effects of collagen have been carried out in horses.

2.42 Collagen – type II

Type II collagen is one of the major connective tissues which gives the joints resilience and support [54]. Its breakdown indicates that OA is advancing [53]. Undenatured type II collagen (UC-II) is better than the denatured form for horses with arthritis [54].

2.42.1 Mode of action

When "UC-II is ingested, stomach acids and enzymes perform a partial digestion of the collagen matrix, resulting in chains of soluble collagen molecules of varying length, containing biologically active epitopes" [54]. These epitopes are though to enter the Peyer's patches and there they trigger the conversion of naive T-cells into T regulatory cells (Treg cells). These Treg cells stay circulating in the blood and upon the recognition of UC-II in the joint cartilage, they release anti-inflammatory mediators (transforming growth factor-beta, IL-4 and IL-10). This mechanism of action is referred to as oral tolerance and is supported by several studies [55]. Through this mechanism, the body separates foreign invaders from useful nutrients [54].

2.42.3 Efficacy

A controlled study was done in horses with a moderate level of osteoarthritis to investigate the effects of UC-II (320 mg, 480 mg and 640 mg which give 80, 120 and 160 mg of active UC-II respectively) and a combination of glucosamine and chondroitin (5.4 g and 1.8 g respectively). The results demonstrated that all doses of UC-II and, to a lesser extent, the glucosamine and CS combination, caused a significant analgesic effect. With that being said, the authors suggested that the best dose for UC-II is 480 mg since the higher dose (640 mg) did not give better results. Furthermore, based on the results of the study, the authors proposed that UC-II is more efficient when given long term [54].

In a controlled study involving arthritic horses, the results suggested that UC-II at a dose of 80-160 mg/kg can help relieve the clinical signs of arthritis [55].

2.43 Silicon (Si)

The following effects of Si may play a role in the establishment and advancement of OA and consequently decrease lameness: elevation of collagen production, reduction of bone resorption and elevation of osteoblast differentiation through which bones develop and mineralise and increase the absorption and utilisation of other minerals, including calcium and boron which are also involved in bone development [56].

2.43.1 Efficacy

A randomised controlled study was done to investigate the effects of silicon supplementation in mature horses. No amelioration in lameness and no effect on cartilage turnover, demonstrated by the fact that there was no rise in the synovial fluid marker levels, was seen in the supplemented horses (0.3 g/100 kg body weight daily) compared to the non-supplemented horses. Based on these results, the authors suggested that these results demonstrate that Si supplementation does not exert the same advantageous effect in older horses compared to younger ones. One reason for this might be that mature horses require a greater amount of Si due to their larger size than younger horses. This is in contrast to past research which demonstrated "reduced carboxy-terminal pyridinoline cross-linked telopeptide region of type I collagen, a bone resorption marker, in Si-supplemented young horses compared with controls" [56].

In a controlled study, horses supplemented with 2.8% sodium zeolite A (SZA) demonstrated elevated plasma silicon levels. Furthermore, the results suggested that the administration of SZA might have an advantageous effect on the horses' work and its duration [53].

2.44 Silica

Humans and animals take up silicon as silica from food and feed. Most of this silica is not used by the body but removed via urine and faeces [57].

The study mentioned under 'Collagen' has investigated the effects of a product containing multiple active ingredients including silica [53]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of silica have been carried out in horses.

2.45 Soybean

Soybean "could have a structure-modifying effect in osteoarthritis by inhibiting cartilage degradation and promoting cartilage repair" [58]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.46 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae (SC) is a beta-glucan (BG). "Beta-glucans are carbohydrates composed of glucose molecules linked together by several different types of chemical linkages, resulting in either a linear or branched structure". The number of side chains of BGs varies, the higher their amount, the more 'biologically active' is the BG. SC has been demonstrated to have a significant number of side chain branches as well as antioxidant properties [59].

2.46.1 Efficacy

Most of the studies carried out in horses are regarding digestion, nonetheless, numerous studies in humans and other species have showed SC's beneficial effects on the immune system. Similarly, SC fermentation products have also been the subject of several studies due to the possible similar effects to SC [59]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.47 Stevia

Stevia is a perennial shrub about which several studies have been run in an attempt to understand its mechanism of action regarding its highly publicised anti-inflammatory effects. However, a study ran in horses contraindicated these findings, as the stevia supplemented horses had elevated levels of TNF-α, IL-6, toll-like receptor 4, IFN- γ in comparison to horses supplemented with corn syrup. Therefore, it might be that stevia is not exclusively for or against inflammation but instead it is an immunomodulator [60].

2.48 Milk thistle (*Silybum marianum*)

The active ingredient in milk thistle is silymarin (SM), a complex consisting of three isomer flavolignans called silybin, silydianin and silychristinin, present in all parts of the plant but mainly in the fruit and seeds. Silybin is the most active and makes up 50-70% of the silymarin complex [61].

SM has poor bioavailability the reason for which could be one of the following: destruction by the gastric juice, low gastrointestinal absorption (20-50%) or low solubility in water. To counteract this, SM has been tested in integrated in several dosage forms, such as "cyclodextrin, salts of polyhydroxyphenylchromanones, soluble derivatives, complexes with phospholipids and liposomal encapsulation". Studies have shown that phospholipid complexes and specifically a silymarin-phosphatidylcholine complex are the most lipid-soluble and therefore, are the best at crossing cell membranes [62].

2.48.1 Efficacy

SM has antioxidant properties most probably manifested through the activation of NF-κB pathway which in turn stimulate a number of enzymatic and non-enzymatic [61]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.49 Rosehip (*Rosa canina*)

The active ingredients which are definitely known to be responsible for rosehip's anti-inflammatory properties are flavonoids and FAs. However, based on the results of *in vitro* studies, there may be some other active ingredients which also possess these properties [63].

2.49.1 Efficacy

In a double blind, controlled study the horses supplemented with rosehip powder (210 grams daily) had increased anti-inflammatory activity shown by the reduction in neutrophil chemotaxis and better performance in races. Furthermore, the staff described the horses as being "more lithe after exercise". However, it was argued that these beneficial effects could be attributed to other reasons. The first reason is that the horses in the treatment group had a worse best record for the race than the horses in the control group, thus, they had more room for improvement. The second reason given was that the results observed might have been influenced by the horses' individual differences [31].

2.49.2 Dosage

Studies showed that rosehip treatment efficacy is positively correlated to the dose [63].

2.50 Meadowsweet (*Filipendula ulmaria*)

Meadowsweet is a herb found in 'natural' substitute products for Bute, a drug used to manage pain and inflammation resulting from training in horses [64]. Care must be taken when supplementing horses with it as it may give a positive result when testing for drugs due to its salicylate content [31].

2.50.1 Efficacy

A study showed that salicylate in willow bark (120-140 mg per day) can act against inflammation and pain. Salicylate is also present at a high concentration in meadowsweet, however, whether it can also exert these actions is yet to be discovered since information regarding its quantity in the herb and bioavailability is missing [37]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.51 Cranberry

Cranberries are a major source of flavonols, mainly quercetin [65].

A controlled study was done in horses to check the bioavailability of 90MX cranberry powder administered in a low dose (200 mg) and high dose (400 g) by measuring the concentrations of six flavonol compounds. The results demonstrated that cranberry powder is bioavailable and that there was no significant difference in the level of maximal concentrations of flavonols recorded in the muscles in the horses supplemented with a low dose in comparison to those supplemented with a high dose [65].

2.51.1 Efficacy

Studies demonstrated that: polyphenols safeguard the endothelium from oxidative and inflammatory markers produced in response to stress, phenolics, including quercetin and cyanidin, present in cranberries were found to be radical scavengers and cranberry has the ability to stop the action of TNF-α but not that of IFN-γ in horses doing extreme exercise [29].

2.52 Quercetin

As previously mentioned, quercetin is a flavonol found in cranberries [65].

A study investigating the bioavailability of pure quercetin (6 grams in 2 litres of water) found that the aglycone form of quercetin is not bioavailable [65]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.53 Eggshell membrane

Natural eggshell membrane (NEM) is a by-product of poultry and a source of collagen, GAGs and protein [66].

2.53.1 Efficacy

A study done to investigate the effect of Steadfast®, NEM® and chelated trace minerals (CTM; zinc, copper and manganese) in horses (50 grams daily) and rats with OA found that the joint supplement elevated the production of cartilage or reduced its breakdown. Furthermore, the combination of NEM® and CTM led to a reduction in pain, swelling and CTXII, a biomarker of cartilage degradation, greater than that provided by NEM® or CTM alone. Therefore, this joint supplement, NEM® and CTM all aid in the maintenance of healthy bones and joints [66]. However, it is not known which of the ingredients is responsible for this effect. Therefore, at the time of my research work, no clinical studies have been carried out in horses.

2.54 Bioflavonoids

Bioflavonoids are natural constituents of fruits, teas, spices, wine and vegetables such as pomegranate, ginger, turmeric, and rosehip. Studies have suggested that due to their action against inflammation, catabolism and oxidation, bioflavonoids may play a role in altering the outcomes of OA disease [67]. Further information regarding them can be found under ‘Citrus’ and ‘Hesperidin’ in this literature review.

2.55 Citrus (*Citrus sinensis*)

Citrus fruits contain nobiletin, a polymethoxylated flavonoid found to be beneficial against OA in animal and human studies. These studies demonstrated that nobiletin: administered at a dose of 16-64 μM can suppress the enzymes (APAMTS -4 and -5) involved in cartilage breakdown in a culture of human synovial fibroblasts, prevents pannus production and joint matrix damage, prevents the synthesis of factors responsible matrix breakdown such as promatrix metalloproteinase and PGE2 in rabbit synovial fibroblasts and articular

chondrocytes respectively, and, stimulates MMP inhibitor, thereby supporting matrix construction in a synovial fibroblasts, macrophages and articular chondrocytes of humans, mice and rabbits respectively [68].

Citrus sinensis (orange) peel also has bioflavonoids, such as polymethoxylated flavones (PMFs). PMFs are known to possess anti-inflammatory and antioxidant properties. PMFs have been found to inhibit TNF-α, IFN-γ, IL-1B and IL-6 expression in several studies mentioned by [68].

A study involving 9 mares supplemented with decaffeinated black tea and orange peel extracts. The latter were found to reduce the levels of IFN-γ, proving their anti-inflammatory properties [20].

At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.56 Hesperidin

Hesperidin is a bioflavonoid mainly found in unripe fruit, such as orange and lemon [69]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.57 Q10

2.57.1 Efficacy

A randomised, controlled study was done to investigate the effects of a supplement consisting of curcumin, *Boswellia*, Q10, glycine propionyl-L-carnitine HCl and D-ribose to horses. The results demonstrated that these active ingredients decrease the activity of pro-inflammatory cytokines and increase the adaptation to exercise [70]. However, it is not known which of the ingredients is responsible for this effect. Therefore, at the time of my research work, no clinical studies have been carried out in horses.

2.57.2 Dosage

There is no data in the literature regarding the dosage required for Q10 to produce its desired effects, however, stated that 800 mg of Q10 daily significantly elevated its levels from baseline [70].

2.58 Berberine

An alkaloid found in the root and bark of several plants. Similar to other phytoconstituents, berberine's bioavailability is problematic [71]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.59 Oat beta glucan

Beta-glucans are a class of polysaccharides present in the cell wall of oats but also yeast, barley, mushrooms and seaweed [72]. In general, supplementation of beta-glucans strengthens the roles of the immune system through the direct triggering of the macrophages, neutrophils and natural killer cells [73]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.60 CBD

CBD is a non-psychoactive compound present in hemp. Nowadays, it is becoming increasingly popular as a substitute for pharmacological drugs in the treatment of various diseases, including but not limited to, arthritis [74].

2.60.1 Efficacy, dosage, side effects

In studies cited carried out in mice and horses, CBD was found to decrease the synthesis of pro-inflammatory cytokines including TNF-α. This demonstrates CBD's potential to exert anti-inflammatory effects in horses [74].

Another study further proved this anti-inflammatory potential as well as its analgesic effect. A mare demonstrating high sensitivity when touched in the shoulder region receiving 500 mg orally of CBD daily markedly ameliorated. However, when the dose was decreased to 250 mg daily, the clinical signs reappeared within a day. The dose was therefore increased back to 500 mg and slowly decreased over the period of two months to a maintenance dose of 150 mg. At this stage, the horse did not show increased sensitivity and the owner reported a 90% improvement [75].

In a study it was found that all horses supplemented with 50 mg of CBD and five of the six horses supplemented with 100 mg of CBD had serum CBD concentration below the minimum detectable limit. This was not the case for the horses supplemented with 250 mg [75].

Therefore, at the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.61 Copper (Cu)

In horses, insufficient intake of copper has been linked to osteochondritis and developmental orthopaedic diseases. Copper is necessary for appropriate collagen formation as the cross-linking of collagen subunits are dependent on it [76].

2.61.1 Efficacy

Studies have demonstrated amelioration in joint health when supplementing chelated trace minerals (CTM) instead of inorganic forms. However, a research study found no significant differences in the results of horses supplemented with CTM (copper, zinc, manganese and cobalt) compared to those supplemented with inorganic trace minerals. These findings are in accordance with other studies. With that being said, a study found that on week 12, the horses supplemented with CTM while undergoing submaximal exercise had a relative increase in CPII relative to C2C. This proves the advantageous effects on joint health [76].
Another study found that CTM (copper, zinc, manganese and cobalt) may aid in ECM turnover when joints are challenged with LPS. This was proven by the rise in type II collagen and a faster increase in aggrecan synthesis [77].
However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of Cu have been carried out in horses.

2.62 Zinc (Zn)

Studies mentioned under ‘Copper (Cu)’ have investigated the effects of CTM, including Zn [76, 77]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of Zn have been carried out in horses.

2.63 Manganese (Mn)

Studies mentioned under ‘Copper (Cu)’ have investigated the effects of CTM, including Mn [76, 77]. However, it is not clear which active substance is responsible for the effects

observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of Mn have been carried out in horses.

2.64 Cobalt (Co)

Studies mentioned under 'Copper (Cu)' have investigated the effects of CTM, including Co [76, 77]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of Co have been carried out in horses.

2.65 Iodine (I)

Molecular iodine exerts the following effects in vertebrate animals: antioxidants connecting "or competing with free radicals for membrane lipids, proteins, and DNA, increasing the expression or activity of antioxidant enzymes or" deactivating pro-inflammatory pathways and modulation of the immune response via its direct action in distinct immune cells [78]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.66 Magnesium (Mg)

Mg possesses anti-inflammatory properties; in fact, when in insufficient levels, it increases the synthesis of IL-1α and IL-6 [79]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.67 Selenium (Se)

Se, a trace mineral, has significant importance in the catalytic activity of GPx, an antioxidant enzyme [80].

A study involved the administration of a supplement containing vitamins E, C and A as well as Cu, Zn and Se daily to horses for 3 months during the racing period. The supplemented horses had significantly elevated vitamin E, vitamin A and selenium levels in the plasma as well as GPx activity and antioxidant capacity when compared to the control horses [80]. At the time of my research work, no clinical studies investigating the individual effects of Se have been carried out in horses.

2.68 Vitamin A

Vitamin A is an endogenous hydrophobic antioxidant [80].

The study mentioned under ‘Selenium (Se)’ has investigated the effects of a supplement containing multiple active ingredients including vitamin A [80]. However, it is not clear which active substance is responsible for the effects observed. Therefore, at the time of my research work, no clinical studies investigating the individual effects of vitamin A have been carried out in horses.

2.69 Vitamin B2

This vitamin has antioxidant properties [81]. Furthermore, it has been demonstrated that it is able to suppress OA in animals [82]. The daily feeding ration should be able to cover the vitamin B2 requirements without the need for oral supplementation [7]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.70 Vitamin B7 / biotin

Biotin is commonly supplemented to horses to better hoof health. Better hoof health decreases the chances of having unevenly loaded joints and therefore, the occurrence of degenerative joint disease as well [83]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.71 Vitamin D

In summer, horses most probably reach their vitamin D requirements with exposure to sunlight. However, it is dangerous to rely on sun exposure in winter in many areas. Therefore, vitamin D is commonly supplemented in feedstuff as a deficiency in it leads to bone problems and swelling of joints [84]. Studies available on vitamin D and drew the following conclusions: vitamin D’s role on the commencement and advancement of OA is poorly understood and that studies do not provide sufficient evidence on this vitamin’s defensive action neither against volume loss in the cartilage nor on radiologic OA commencement [85]. Therefore, at the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.71.1 Dosage

The NRC recommends 136-364 IU vitamin D with every pound of feed. This value can be increased in case of low levels of calcium and phosphorus or an inappropriate ratio between them. However, if the imbalance of calcium and phosphorus is too drastic, vitamin D supplementation will not be beneficial [84].

2.72 Arginine

Arginine is an amino acid necessary for the production of "ornithine, polyamines, proline, glutamine, creatine, agmatine and nitric oxide". These molecules are crucial for: cellular production, movement and remodelling, formation and dilution of blood vessels and cell signalling pathways [86]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.72.1 Dosage

Its dietary requirement is unknown in horses [86].

2.73 Glycine

It is an essential amino acid which, along with proline, is found in high concentrations in collagen supplements. These are the two amino acids crucial for the stabilisation and regeneration of the cartilage tissue [55]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.74 Proline

As discussed under 'Glycine', proline is found in high concentrations in collagen supplements as it is crucial for the stabilisation and regeneration of the cartilage tissue [55]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.75 Coconut oil

This oil contains high levels of saturated fat; therefore, it should not be given to horses in any form [87]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.76 Black pepper (*Piper nigrum*)

Traditionally, black pepper was used to treat inflammatory conditions. These anti-inflammatory properties are also backed up by modern science. The 5th Century Syriac Book of Medicines recommends the use of pepper to treat joint pain [88]. It is also recommended for the treatment of arthritis [89]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.77 Ascorbyl

Ascorbyl is found in several different forms. Ascorbyl palmitate, a derivative of ascorbic acid soluble in lipids, has a better bioavailability than ascorbic acid, ascorbyl stearate and calcium ascorbyl 2 monophosphate [18]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.78 Rosemary (*Salvia rosmarinus*)

This herb is traditionally used to alleviate pain and inflammation, including arthritis [90]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.79 Oregano (*Origanum vulgare*)

This herb contains rosmarinic acid which has been demonstrated to possess antioxidant, anti-inflammatory and immunomodulatory properties [91]. Apigenin, another constituent of oregano, also possesses antioxidant and anti-inflammatory properties [92]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.80 Ginkgo (*Ginkgo biloba*)

EGb761 is a standardised extract of Ginkgo biloba leaves containing the active ingredients quercetin and kampferol. These active constituents exert anti-inflammatory effects in human chondrocytes manifested via the blocking of toll-like-receptor 4 [93]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.81 Blackberry powder

Blackberries contain the active ingredients anthocyanins, ellagitannins and ellagic acid which possess anti-inflammatory and antioxidant properties [94]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.82 Elderberry powder

Elderberry can act against oxidation and improve the functioning of the immune system [95]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.83 Raspberry powder

The skin of raspberries contains resveratrol, a chemical which has antioxidant, anti-inflammatory and anti-OA effects [96]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.84 *Bacillus subtilis*

Pure plipastatins and/or a mixture of plipastatins and surfactins, families of lipopeptides belonging to B. *subtilis* strains, possess antioxidant properties [97]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.85 Milk protein

Milk protein is a major natural supplement used to manage OA in veterinary medicine [55]. With that being said, at the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.86 Dimethylglycine (DMG)

DMG possesses anti-inflammatory and antioxidant properties [98]. At the time of my research work, I could not find any information regarding the beneficial effects on equine joints.

2.87 Boron

A study found that boron supplementation (3 mg/25 kg) to arthritic dogs, horses and cattle led to amelioration within 2-4 weeks. However, this observation has not been proven with controlled experiments and this information was regarded to be weak by the authors [99].

3. Materials and Methods

Three of the most common search engines were used to check for the quality of the information provided to the consumers, for the presence of a complete list of ingredients and to evaluate the product quality of the nutraceuticals available on the market – these are: Google, Yahoo and Bing!

The key words "oral joint supplements for horses" were used to conduct the search and the first 30 results that come up on each search engine were evaluated to find websites which are selling these products.

Websites which a) sell products directly to the customer, b) do not require the consumer to create an account to see the aforementioned list and c) are in english, were be included.
On the other hand, websites which a) have broken links, b) do not categorise products according to what disease they're targeting, c) are articles, d) are research papers, e) are marketplaces and f) are advertisements were excluded.

If a website did not contain the ingredient list of a certain product, the website of the product's manufacturer was used to collect the ingredients' list (and other missing information).

To analyse scientific evidence of the efficacy of these supplements, a Google Scholar search was carried out **on ingredients shared by at least 3 products** using the following key words "[name of NC] – oral joint supplements for horses" or "[name of NC] + equine joint" to find work published in the last 15 years. Furthermore, Equine Applied and Clinical Nutrition published in 2013 was used a source of evidence-based information.

The literature analysis focused on the findings regarding the efficacy of these ingredients in treating joint diseases, the method of administration, the indications for specific diseases and the possible side effects. An ingredient was accepted as an effective one if any equine related *in vivo* study was published which confirmed its efficacy.

4. Results

Out of the 90 websites used for this research, 21 (23.33%) websites were useful. From these 21 websites, a total of 321 products were collected which contained a total of 344 active ingredients.

The 10 most frequently used ingredients in the products were: MSM (found in 202 (62.93%) products), glucosamine (found in 186 (57.94%) products), chondroitin (found in 138 (42.99%) products), vitamin C (found in 136 (42.37%) products), hyaluronic acid (found in 134 (41.74%) products), Mn (found in 84 (26.17%) products), Zn (found in 74 (23.05%) products), *Yucca* (found in 68 (21.18%) products), Cu (found in 63 (19.63%) products) and *Boswellia* (found in 58 (18.07%) products).

Out of the 344 active ingredients, 164 (47.67%) active ingredients used for more than 3 products and thus, researched. From these 164 active ingredients, 11 (6.71%) active ingredients, summarised in **tables 1 to 8** had scientific evidence *in vivo* in equine which supported their claimed effects. 231 (71.96%) products were found to contain the ingredients which were deemed to have conflicting results. 153 (44.48%) active ingredients, summarised in **tables 9 to 31** had no scientific evidence *in vivo* in equine proving their claimed effects. Therefore, 180 (52.33%) active ingredients, summarised in **tables 32 to 63**, were not researched since they were found in less than 3 products. 33 (10.28%) products were considered to be 'absolutely perfect' as they only contained active ingredients for which there is scientific evidence *in vivo* in equine to support their use. An additional 2 products could potentially be absolutely perfect as well; however, they contained ingredients which were in less than 3 products and thus, not researched.

11 (3.4%) out of the 321 products used in this thesis did not have an ingredient list. Furthermore, 56 (17.45%) of the products that did have an ingredient list did not provide accurate information as they did not provide information regarding the amount of active ingredients in the product.

Table 1: ingredients which provide building blocks with scientific evidence *in vivo* in equine

name of active ingredient	**effective in vivo in eq?**	**dose**	**mechanism of action**	**number of products containing it**	**number of publications**
chondroitin	yes	2-6 grams/day [2]	slowing down of cartilage breakdown; provision of building blocks required for the synthesis of ECM; decrease the levels of PGE2 in synovial fluid, production and distribution of MMP-3 and glycosaminoglycans	138	4
collagen - type II	yes	120 mg [54] 80-160 mg/kg [55]	analgesic effect, relieves clinical signs of arthritis	12	3
hyaluronic acid	conflicting results	100 mg/day [9]	decreases effusion	134	4
MSM	conflicting results	8 mg/kg [15] 4.8 grams [2]	protective effect in exercise related inflammation and oxidative damage	202	4

Table 2: elements with scientific evidence *in vivo* in equine

name of active ingredient	effective in vivo in eq?	dose	mechanism of action	number of products containing it	number of publications
Si	conflicting results	N/A	elevates collagen production, reduces bone resorption and elevation of osteoblast differentiation, increases the absorption and mineralisation of other minerals involved in bone development	10	2

Table 3: vitamins with scientific evidence *in vivo* in equine

name of active ingredient	effective in vivo in eq?	dose	mechanism of action	number of products containing it	number of publications
vitamin C	yes	50 g rosehip powder, a source of vitamin C [18]	decreases oxidative stress	136	4

Table 4: animals and animal parts with scientific evidence *in vivo* in equine

name of active ingredient	effective in vivo in eq?	dose	mechanism of action	number of products containing it	number of publications
green lipped mussel (*Perna Canaliculus*) extract	conflicting results	25 mg/kg [17]	analgesic and anti-inflammatory properties, bettered joint flexion	17	4

Table 5: fruit with scientific evidence *in vivo* in equine

name of active ingredient	effective in vivo in eq?	dose	mechanism of action	number of products containing it	number of publications
Rosehip (*Rosa canina*)	yes	210 grams [31]	anti-inflammatory properties	7	2

Table 6: seeds with scientific evidence *in vivo* in equine

name of active ingredient	effective in vivo in eq?	dose	mechanism of action	number of products containing it	number of publications
Celery (*Apium graveolens*) seed	yes	30 g [47]	analgesic effect	7	1

Table 7: stillbenoid with scientific evidence *in vivo* in equine

name of active ingredient	effective in vivo in eq?	dose	mechanism of action	number of products containing it	number of publications
resveratrol	conflicting results	1000 mg [21] 2000 mg [22]	reduced level of lameness, increase in antioxidant enzyme levels, decrease in oxidative stress marker MDA, anti-inflammatory properties	19	4

Table 8: other ingredients with scientific evidence *in vivo* in equine

name of active ingredient	**effective in vivo in eq?**	**dose**	**mechanism of action**	**number of products containing it**	**number of publications**
ASU	yes	N/A	decrease in the synovial membrane inflammation and in the cartilage disease score	6	1

Table 9: ingredients which provide building blocks without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
CMGP (cartilage matrix glycoprotein)	3
collagen	36
collagen - type I	4
collagen - type III	3
glucosamine	186
protein hydrolysate (bovine)	3

Table 10: elements without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
boron	10
Ca	32
Co	6
Cr	4
Cu	63
Fe	5
I	6
Mg	21
Mn	84
K	7
P	9
S	9
Se	12
Se-yeast	7
Zn	74

Table 11: compounds without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
berberine	3
choline	7
shark cartilage	3

silica	16
sodium chloride	4

Table 12: vitamins and provitamins without scientific evidence *in vivo* in equine

name of active ingredient	**number of products containing it**
beta-carotene	4
vitamin A	18
vitamin B1	18
vitamin B2	18
vitamin B3	17
vitamin B5	17
vitamin B6	31
vitamin B7 / biotin	27
vitamin B9	13
vitamin B12	21
vitamin D	16
vitamin E	57
vitamin K3	3

Table 13: amino acids and their derivatives without scientific evidence *in vivo* in equine

name of active ingredient	**number of products containing it**
arginine	7
aspartic acid	5
creatine	11
glutamine	23
glycine	14
dimethylglycine (DMG)	4
histidine	3
isoleucine	5
leucine	5
lysine	41
methionine	44
phenylalanine	13

proline	11
serine	4
threonine	13
tyrosine	4
valine	3

Table 14: bacteria without scientific evidence *in vivo* in equine

name of active ingredient	**number of products containing it**
Aspergillus niger	5
Aspergillus oryzae fermentation product	7
Bacillus licheniformis	6
Bacillus subtilis	13
Bifidobacterium longum	7
Lactobacillus acidophilus	13
Lactobacillus casei	7
Lactobacillus lactis	4
Enterococcus faecium	10
Enterococcus lactis	3

Table 15: prebiotics without scientific evidence *in vivo* in equine

name of active ingredient	**number of products containing it**
arabinogalactan	4
fructooligosaccharides	7
mannanooligosaccharides	6

Table 16: trees, plants and shrubs without scientific evidence *in vivo* in equine

name of active ingredient	**number of products containing it**
aloe vera	6
american wintergreen (*Gaultheria procumbens*)	3
wolf's bane (*Arnica montana*)	3
arishta (*Berberis aristata*)	3
astragalus (*Astragalus membranaceous*) powder	3
punarnava (*Boerhaavia diffusa*)	3

Boswellia (*Boswellia serrata*)	58
burdock (*Arctium lappa*)	4
cat's claw (*Uncaria tomentosa*)	9
chamomile (*Matricaria chamomilla*)	4
chia (*Salvia hispanica*)	3
citrus (*Citrus sinensis*)	20
dandelion (*Taraxacum officinale*)	8
devils claw (*Harpagophytum procumbens*)	37
feverfew (*Tanacetum parthenium*)	8
gale of the wind (*Phyllanthus niruri*)	3
garlic (*Allium sativum*)	5
ginger (*Zingiber officinale*)	14
ginkgo (*Ginkgo biloba*)	4
golden rod (*Solidago*)	5
holy basil (*Ocimum tenuiflorum*)	4
Horseradish (*Armoracia rusticana*)	4
Liquorice (*Glycyrrhiza glabra*)	7
makoi (*Solanum nigrum*)	3
marshmallow (*Althaea officinalis*)	7
meadowsweet (*Filipendula ulmaria*)	8
milk thistle (*Silybum marianum*)	4
nettle (*Urtica dioica*)	8
oregano (*Origanum vulgare*)	4
phellodendron (*Phellodendron amurense*) bark extract	3
black pepper (*piper nigrum*)	8
rosemary (*Salvia rosmarinus*)	5
slippery elm (*Ulmus rubra*)	4
stevia (*Stevia rebaudiana*)	4
Comfrey (*Symphytum officinale*)	3
Turmeric (*Curcuma longa*)	51
white willow (*Salix alba*)	15
Yucca (*Yucca shidigera*)	68

Table 17: algae without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
seaweed	3

Table 18: fruit without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
apple	8
apricot powder	3
bilberry	4
blackberry powder	3
blueberry	6
cherry powder	3
cranberry	7
elderberry powder	3
peach powder	3
raspberry powder	3
strawberry powder	3

Table 19: seeds without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
Fenugreek (*Trigonella foenum-graecum*) seed	7
grape seed extract	25
psyllium seed husk	4

Table 20: oils without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
coconut oil	4
cod liver oil	4
soya bean oil	3

Table 21: fungi without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
Saccharomyces cerevisiae	25

Table 22: enzymes without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
bromelain	17
pancreatin 8x	3
pancrelipase	4
superoxide dismutase (SOD)	9

Table 23: carbohydrates without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
anhydrous glucose	3
dextrose	4
maltodextrin	3

Table 24: alcohol without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
glycerol	4

Table 25: food products without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
soybean	6

Table 26: phospholipids without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
lecithin	8

Table 27: fatty acids without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
cetyl myristoleate	12
omega-3 fatty acids	56
omega-6 fatty acids	24
omega-9 fatty acids	9

Table 28: flavonoids without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
bioflavonoids	5
hesperidin	6
quercetin powder	6

Table 29: antioxidants without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
alpha lipoic acid	5
antioxidants (plant based)	3
Q10	4

Table 30: soluble fibre without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
oat beta glucan	4

Table 31: other ingredients without scientific evidence *in vivo* in equine

name of active ingredient	number of products containing it
ascorbyl	4
bio active whey	4
eggshell membrane	3
kaolin	4
milk protein	7
SAMe (S-adenosylmethionine)	4

Table 32: ingredients which provide building blocks found in less than 3 products

name of active ingredient	number of products containing it
chondrocyte peptides	1
collagen – type V	2
collagen – type X	2
collagen – type B	1
collagen – bovine	1
mucopolysaccharides	2

tendocyte/ligamentocyte peptides	1

Table 33: elements found in less than 3 products

name of active ingredient	number of products containing it
Cr-yeast	1
sodium copper chlorophyllin	1

Table 34: compounds found in less than 3 products

name of active ingredient	number of products containing it
BHT	1
bioperine	1
fumaric acid	2
mono silicic acid	2
omicha	1
para-amino benzoic acid (PABA)	2

Table 35: vitamins found in less than 3 products

name of active ingredient	number of products containing it
vitamin B	2
vitamin D3	1
vitamin K	2
vitamin K1	2
vitamin K2	2

Table 36: amino acids and their derivatives found in less than 3 products

name of active ingredient	number of products containing it
alanine	2
acetyl-L-carnitine	1
asparagine	1
cysteine	1
N-acetyl cysteine	1
ornithine	1
L-ornithine HCl	1

taurine	1
tryptophan	1
L-valine	1

Table 37: bacteria found in less than 3 products

name of active ingredient	**number of products containing it**
Bifidobacterium animalis	1
Bifidobacterium thermophilum	2
Lactobacillus plantarum	2
direct fed microbials	1

Table 38: prebiotics found in less than 3 products

name of active ingredient	**number of products containing it**
inulin	1

Table 39: animals and animal parts found in less than 3 products

name of active ingredient	**number of products containing it**
European sourced porcine plasma	1
cartilago suis	2
placenta suis	2
embryo suis	2
funiculus umbilicalis suis	2

Table 40: trees, plants and shrubs found in less than 3 products

name of active ingredient	**number of products containing it**
black cutch (*Acacia catechu*)	2
aconite (*Aconitum napellus*)	1
amla (*Phyllanthus emblica*)	2
yellow wild indigo (*Baptisia tinctoria*)	1
deadly nightshade (*Atropa belladonna*)	1
common daisy (*Bellis perennis*)	1
barberry (*Berberis vulgaris*) root extract	2
black cohosh (*Actaea racemosa*) root	1

white bryony (*Bryonia alba*)	2
pot marigold (*Calendula officinalis*)	1
camelina (*Camelina sativa*)	2
chaparral (*Larrea tridentata*)	1
chuchuhuasi (*Maytenus laevis*) bark extract	2
Cinnamon (*Cinnamomum verum*)	1
Coffee (*Coffea cruda*)	1
downy hemp nettle (*Galeopsis segetum*)	1
bittersweet nightshade (*Solanum dulcamara*)	2
coneflower (*Echinacea purpurea*)	2
edible stemmed vine (*Cidrus quadrangularis*)	2
field horsetail (*Equisetum arvensis*)	1
cleavers (*Galium aparine*)	1
ginseng (*Panax ginseng*)	1
goldenseal (*Hydrastis canadensis*)	1
goldthread freeze dried root extract (*Coptis*)	2
ground barley grass (*Hordeum vulgare*)	1
guduchi (*Tinospora cordifolia*)	2
gurmar (*Gymnema sylvestre*)	2
jiaogulan (*Gynostemma pentaphyllum*) leaf	1
american witch hazel (*Hamamelis virginiana*)	1
hawthorn (*Crataegus monogyna*)	2
hops (*Humulus lupulus*)	2
st john's wort (*Hypericum perforatum*)	1
saint ignatius bean (*Ignatia amara*)	1
bay laurel (*Laurus nobilis*) leaf	1
marsh labrador tree (*Ledum palustre*)	2
houpu magnolia (*Magnolia officinalis*)	2
moschus (*Abelmoschus moschatus*)	1
noni (*Morinda cirtrifolia*)	1
strychnine (*nux vomica*)	1
olive (*Olea europaea*) leaf	1
purple passionflower (*Passiflora incarnata*)	1

pink trumpet (*Handroanthus impetiginosus*)	1
poplar (*Populus*)	2
purple osier (*Salix purpurea*)	1
red clover (*Trifolium pratense*)	1
siberian rhododendron (*Rhododendron chrysanthum*)	2
butcher's broom (*Ruscus aculeatus*)	1
common rue (*Ruta graveolens*)	1
rhus tox (*Toxicodendron pubescens*)	2
bloodroot (*Sanguinaria canadensis*)	2
chinese skullcap (*Scutellaria baicalensis*)	1
marsh skullcap (*Scutellaria galericulata*) powder	1
siberian ginseng (*Eleutherococcus senticosus*) root	1
sea buckthorn (*hippophae rhamnoides*)	1
ku shen (*Sophora flavescens*) extract	2
Spearmint (*Mentha spicata*)	1
valerian (*Valeriana officinalis*)	2
false helleborine (*Veratrum album*)	1
common grape vine (*Vitis vinifera*)	1
winter cherry (*Withania somnifera*)	2
yarrow (*Achillea millefolium*)	2
corn (*Zea* mays)	1

Table 41: algae found in less than 3 products

name of active ingredient	**number of products containing it**
algae rich in omegas	1
dried algae (Aurantiohytrium SP)	1
spirulina	1
kelp	2
Lithothamnium calcareum	2

Table 42: herbs found in less than 3 products

name of active ingredient	number of products containing it
arjuna	2
basil	2
cleaver herb	2
gotu kola herb	1
mint	1
thyme	1
vervain herb powder	1
Viola tricolor herba	1

Table 43: fruit found in less than 3 products

name of active ingredient	number of products containing it
acai berry extract	2
acerola	1
Ananas comosus	1
Uva ursi	1
black currant	1
goji berry extract	2
mango	2
olive extract (fruit)	1
orange peel	2
papaya	2
pomegranate extract	2
tart cherry powder	2

Table 44: vegetables found in less than 3 products

name of active ingredient	number of products containing it
cabbage powder	1
dried carrot	1

Table 45: seeds found in less than 3 products

name of active ingredient	number of products containing it
ground hulled sunflower seed	1

Table 46: oils found in less than 3 products

name of active ingredient	number of products containing it
bluegum essential oils	1
clove essential oils	2
evening primrose oil	2
fish oil (anchovy)	2
mussel oil	1
organic chia seed oil	1
paraffin oil	1
vegetable oil	1
wheat gem oil	1

Table 47: fungi found in less than 3 products

name of active ingredient	number of products containing it
Cordyceps sinensis	1
Hericium erinaceus	1

Table 48: pigments found in less than 3 products

name of active ingredient	number of products containing it
rutin	2
phycocyanin	2

Table 49: carbohydrates found in less than 3 products

name of active ingredient	number of products containing it
inositol	2
sorbitol	2
sucrose	2

Table 50: alcohols found in less than 3 products

name of active ingredient	number of products containing it
propylene glycol	1

Table 51: food products found in less than 3 products

name of active ingredient	number of products containing it
cayenne pepper	1
corn starch	1
wheat middlings	1
wheat bran	1

Table 52: medications found in less than 3 products

name of active ingredient	number of products containing it
CBD	5
inhibitol	1

Table 53: lipids found in less than 3 products

name of active ingredient	number of products containing it
gamma oryzanol	1

Table 54: phospholipids found in less than 3 products

name of active ingredient	number of products containing it
phospholipids (phosphatidylcholine)	1

Table 55: fatty acids found in less than 3 products

name of active ingredient	number of products containing it
celadrin (fatty acid complex)	1
free fatty acids	1
olive fatty acids	1

Table 56: flavonoids found in less than 3 products

name of active ingredient	number of products containing it
naringenin	1

total catechins (from green tea extract)	1

Table 57: polyphenols found in less than 3 products

name of active ingredient	number of products containing it
Polyphenols (*Camelina sinensis*)	1

Table 58: terpenes found in less than 3 products

name of active ingredient	number of products containing it
astaxanthin	1

Table 59: phenylethanoid found in less than 3 products

name of active ingredient	number of products containing it
hydroxytyrosol	1

Table 60: isoflavones found in less than 3 products

name of active ingredient	number of products containing it
genistein	1

Table 61: soluble fibre found in less than 3 products

name of active ingredient	number of products containing it
pectin	1

Table 62: registered formulas found in less than 3 products

name of active ingredient	number of products containing it
restaurex™ proprietary stem cell activator	1
regenerex MP™ a proprietary formula	1
butiPEARL® Z EQ	1
acti-meal® proanthocyanidins	1
Melofeed® superoxide dismutase	1
Epiitalis®	1

Table 63: other ingredients found in less than 3 products

name of active ingredient	number of products containing it
cane molasses	2
dried whey	1
green tea	2
gum resin extract	1
honey	1
l-carnosine	1
rice bran	2
sepiolite	2
whey protein isolate	2

5. Discussion

Only 21 out of 90 websites (23.33%) were useful for this research as the searches linked to articles, research papers and marketplaces. A total of 321 products and 344 active ingredients were collected. From these 344 active ingredients, 164 (47.67%) active ingredients were present in more than 3 products. These results demonstrate the wide availability and variety of equine oral joint supplements on the market.

Even though glucosamine, Mn, Zn, Yucca, Cu were 5 of the 10 most commonly used ingredients in the products, no scientific evidence is available *in vivo* in equine investigating the effects of the individual ingredients. There is no scientific evidence investigating Boswellia, another one of the 10 most commonly used ingredients, *in vivo* in equine.

The results demonstrate that only 11 (6.71%) of the active ingredients are scientifically proven to act *in vivo* in equine. These ingredients demonstrated several different mechanisms of action through which they act on joints.

Ingredients in joint supplements possess anti-inflammatory properties, including action against PGE2. PGE2 increases in the beginning phase of OA and is involved in the synthesis of the enzymes responsible for damaging the cartilage [41]. Effusion is the build-up of synovial fluid in the joint as a result of the inflammatory processes associated with OA [100]. Therefore, this is why ingredients having the ability to decrease swelling are also present in joint supplements. According to my literature review the following ingredients have anti-inflammatory effects: chondroitin, hyaluronic acid, MSM, green lipped mussel, rosehip, resveratrol and ASU.

They also possess antioxidant properties. In the case of degenerative joint diseases, reactive oxygen species (ROS) are synthesised in excessive amounts and cause joint structure damage [22]. According to my literature review the following ingredients have antioxidant effects: MSM, vitamin C and resveratrol.

The pathophysiological process of OA results in cartilage damage [101]. It leads to increased breakdown by MMP and aggrecanase. Moreover, in the beginning stages of OA, GAG levels are elevated and then decrease later on [102]. This is why products contained ingredients which promote cartilage reparation, or which have a chondroprotective effect.

According to my literature review the following ingredients provide collagen or protect the cartilage: chondroitin, collagen – type II, Si and ASU.

Spontaneous joint diseases are frequent clinical problems which lead to lameness and OA accounts for 60% of it [103]. Furthermore, through personal experience obtained by following the work of veterinarians in Malta, horses are less willing to have their limbs flexed when performing flexion tests and try to pull it away. Most of these horses usually show signs of lameness when they are then trotted. Therefore, it is no surprise that the ingredients found in the products target lameness and joint flexion.

OA also causes pain [103]. According to my literature review were found to have an analgesic effect: collagen – type II, green lipped mussel and celery seed.

Certain ingredients were supported by *in vitro* studies on equine models (as well as laboratory animals and/or cell cultures); however, this efficacy does not directly translate to *in vivo* efficacy as there are several factors which play a role in determining the efficacy of an active ingredient including the bioavailability and protection from digestion. In other cases, there were studies which proved the efficacy of these active ingredients in laboratory animals and/or humans. Nonetheless, once again, these cannot be extrapolated to equine, due to species differences. Furthermore, the *in vivo* studies ran in equine often tested a product containing multiple active ingredients or herbal blends making it difficult to determine which ingredient is responsible for which effect, if it is responsible for any effect that is. Moreover, 231 (71.96%) products contained ingredients which had conflicting results. Therefore, all these points highlight the need for more clinical trials in equine. This means that 153 (44.48%) active ingredients did not have proof of efficacy *in vivo* in equine.

The doses recommended by the websites of the 33 ‘absolutely perfect’ products were compared to those recommended by the scientific studies and/or those of the American Association of Equine Practitioners (AAEP) [104]. 10 products had an appropriate dosage recommendation, 16 products had inappropriate feeding instructions as the weight of the horse for which the dose recommended was not specified, 2 products had a suboptimal dosage recommendation, and 5 products recommended a higher dose than that by the scientific studies and/or AAEP.

Even though OJSs are so popular since they account for a third of the sales of supplements in the USA [5], the supplements on the market are still lacking crucial information regarding

the ingredient list and feeding instructions. Due to the absence of the amounts of active ingredients as well as appropriate feeding instructions, one cannot conclude whether these products are effective even if they contain scientifically proven ingredients as the doses inside the product and/or those provided blindly by horse owners could be suboptimal.

The ACCLAIM system is a useful concept to determine whether a product is safe and effective. However, it was not used for the evaluation of the products and ingredients in this thesis.

The letter ‘A’ in ACCLAIM refers to whether one recognises the company name which manufactures the product as well-known companies are usually known to provide educational material compared to recently established companies [5]. However, I find this to be a biased and unfair approach.

The letter ‘C’ refers to clinical research carried out by the companies manufacturing these products [5]. However, once again I find that this might be a biased approach since researchers being funded by a company might have conflict of interest.

A limitation of this thesis is that only those active ingredients which were found in at least three products were researched. This led to 180 (52.33%) ingredients not being researched. Therefore, there might be other ingredients which are efficacious on the joints yet were not covered in the review.

6. Summary

This thesis aimed at checking the availability of scientific evidence *in vivo* in equine regarding the active ingredients found in oral joint supplements as well as the appropriate labelling with complete lists of ingredients.

The results demonstrated that the majority of active ingredients used in the products for this literature review are not supported by scientific evidence *in vivo* in equine. Furthermore, some of the active ingredients which were researched had conflicting results. However, some significant positive results regarding certain active ingredients were obtained which proved that oral joint supplements could act against inflammation, oxidative processes, and cartilage breakdown. Not only this, but certain active ingredients were also found to protect the cartilage and promote its building. Certain ingredients were researched as part of blends or products without specifying the individual effects of the active ingredients. Other ingredients were researched *in vivo* in humans or laboratory animals; however this efficacy cannot be extrapolated to horses since they are a different species. *In vitro* studies were also available but, once again, the horse is a living animal whose biological processes can affect the efficacy of products. Therefore, further research is necessary to prove that these ingredients are effective *in vivo* in equine so that horse owners and veterinarians can make informed decisions. Nutraceuticals, not being a food nor a drug, do not need to abide to any of the regulations set for these categories. The results proved this as the investigated products were not always appropriately labelled and did not always contain a complete ingredient list.

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Thesis progress report for veterinary students

Name of student: Tamara Abela

Neptun code of the student: HDYCWH

Name and title of the supervisor: Dr Letícia Moravszki

Department: Department of Animal Nutrition and Clinical Dietetics

Thesis title: Review of oral joint supplementation for horses sold on the internet: analysis of provided information and scientific evidence

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	2	7	Topic selection	
2.	2023	3	14	Rules of thesis writing, practical advices, Literature review main focus points	
3.	2023	4	11	Discussion of literature review and plan for searching work	
4.	2023	5	2	Check of product searching, clarification of problematic parts, selection of websites	
5.	2023	5	23	Check of the product list and discussion of ingredients collection	

Grade achieved at the end of the first semester: 5

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	9	2	Check of ingredients list, selection of ingredients for further investigation	
2.	2023	9	15	Discussion of scientific searching methods and type of research publications	
3.	2023	9	28	Selection of collected research articles, discussion of questionable parts	
4.	2023	10	9	Review and evaluation of the entire research, litreture analysis, descritpitve analysis of the results	

5.	2023	10	20	Discusson of methods, results, disscussion, summary, introduction and abstract, writing rules	

Grade achieved at the end of the second semester: 5

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

dr. Kovacsi Leti
.....
signature of the supervisor

Signature of the student: .....F.Abel.....

Signature of the secretary of the department: ...Bakit Ana.....

Date of handing the thesis in...2023.11.05.....

ÁLLATORVOSTUDOMÁNYI EGYETEM
Állatnevelési, Takarmányozástani és Laboratóriumi Állattudományi Intézet
1787
BUDAPEST

University of Veterinary Medicine Budapest

Institute for Animal Breeding, Nutrition and Laboratory Animal Science

I hereby confirm that I am familiar with the content of the thesis entitled *„Review of oral joint supplementation for horses sold on the internet: analysis of provided information and scientific evidence"* written by **Tamara Abela** (HDYCWH) which I deem suitable for submission and defence.

Budapest, 03. November 2023.

ÁLLATORVOSTUDOMÁNYI EGYETEM
Állatenyésztési, Takarmányozástani és Laborállat-tudományi Intézet
1787
BUDAPEST

dr. Moravszki Letícia
supervisor name and signature

Department of Animal Nutrition and Clinical Dietetics

Cím: 1078 Budapest, István utca 2.
Postacím: 1400 Budapest Pf. 2.
Tel.: +36-1-4784-119 | e-mail: wagenhoffer.zsombor@univet.hu