

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



---

## Vitamin K2 in Animal Health: An Overview

---

Jayde O'Neil, Bethany Scarrott,  
Ragnhild Aven Svalheim, Jonathan Elliott and  
Stephen J. Hodges

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/63901>

---

### Abstract

The role of vitamin K in animal health has not received much attention. Vitamin K studies have, for the most part, addressed the use of animals in the investigation of vitamin K physiology and pathophysiology, often using the rodent as a model system. However, vitamin K performs the same role in animals as it does in man and there are areas, such as animal nutrition, where a better understanding of animal requirements in general, and with ageing, could benefit animal health and continued well-being.

**Keywords:** vitamin K2, vitamin K3, menadione, coprophagy, UBIAD1

---

### 1. Introduction

The post-translational gamma-carboxylation of proteins by vitamin K is common across the animal kingdom, yet the organisms most studied for the relationship between this vitamin in health and disease are predominantly man and the rat.

There are several forms of vitamin K: a single vitamer from plants, vitamin K1, or phylloquinone, and a family of K2 vitamins, the menaquinones, which are distinguished by the number of isoprene units in their side chain at position 3 of the 2-methyl-1,4-naphthoquinone moiety. All the long side-chain menaquinones are derived from bacterial sources. Menaquinone-4 is found less frequently in bacteria, those species where it has been reported as a major menaquinone are usually extremophiles and microaerophiles [1–3], and it may be the evolutionary hinge between vitamin K1 using phytoplankton and photosynthetic cyanobacteria [4]. In contrast to bacteria-mediated generation of biologically functional vitamin K2, it is also

---

possible for the specific form of vitamer, menaquinone-4, to be synthesized in animal tissues from vitamin K3 [5–7]. It is also worth noting that, in the rat, vitamin K1 can be converted to menaquinone-4 without the need for bacterial mediators [8–10].

The general understanding of vitamin K in the physiology and pathophysiology of animal health and well-being is less well developed than it is for man. As with man, overt vitamin K deficiency is rare in animals and several examples will be identified. It is clearly not possible to cover all animal species in this overview, and we have selected a reasonably broad range of important agricultural and companion animals.

With respect to captive collection species, although not discussed here as there is very limited information available, the general concepts of vitamin K in the health and well-being of these animals are open to review. This is particularly pertinent to the conservation programmes to support endangered animal breeding projects, as vitamin K2 is beginning to be highlighted as an essential factor in embryogenesis.

For the purposes of this overview, animals can be broadly divided into herbivores and omnivore/carnivores. Common sense would suggest that the former group would be considered to derive most of their vitamin K from the plant vitamer, vitamin K1. We will demonstrate that this is not a simple extrapolation to food source and that vitamin K2 congeners can, in some cases, be at least as dominant a source of vitamin K. The latter grouping of animals is diverse, and we will develop the hypothesis that the vitamin K2 congeners are a dominant form of vitamin K that makes a significant contribution to maintaining the vitamin K status of these animals.

Investigation into the feeding practices for domesticated animals, both companion and agricultural animals, demonstrates that there are several examples where nutritional developments of animal feeds have defaulted into the use of vitamin K3, even though this naphthoquinone compound is unable to participate directly in the gamma-carboxylation of vitamin K-dependent proteins [11]. The value of vitamin K3 in animal nutrition may be derived, in part, from its role as a bacterial growth factor [12–14], where it is converted into vitamin K2 [15]. Many bacteria, particularly the gram-positive bacteria, contain vitamin K2 as their major quinone [16–18]. There are indications that vitamin K2 derived from intestinal bacteria contribute to the vitamin K status of some animals, such as ruminants, more than other animals, and this will be discussed in more detail in the relevant sections.

The following sections will discuss where animals derive their source, or sources, of vitamin K and, where known, what vitamin K-related health pathologies have been described in animals.

## **2. Avian vitamin K requirements**

Given that the original identification of vitamin K was made in the chicken by Henrik Dam [19, 20], it is appropriate to begin this overview by considering avian species. The early work of Dam demonstrated that chicks fed a diet designed to be deficient in sterols, following the non-

polar solvent extraction of their feed, developed subdural or muscular hemorrhage and presented with a retardation in their blood clotting time. Subsequent experiments demonstrated that Inclusion of other essential nutrients, which were known at that time, did not prevent hemorrhage, suggesting the existence of a new vitamin.

Extending his investigations, Dam determined that geese and ducks were also susceptible to the development of clotting problems, but pigeons and canaries appeared to be much less prone to develop hemorrhagic problems [21].

Due to the enormous value of avian species in human nutrition, vitamin K sufficiency is an important subject. Furthermore, the more recent suggestion of a role for vitamin K in skeletal biology also promotes the understanding of adequate vitamin K nutrition in avian species, such as the turkey, which is prone to suffer from skeletal health problems [22] and which has considerable economic consequences [23]. Similarly, broiler chickens, with their rapid growth requirements, also have skeletal health problems [24]. The vitamin K in the feed for hens to offset skeletal health problems has been investigated, and there is some benefit from 8 to 10 mg/Kg feed of vitamin K3 [25, 26]. A more recent study [27] has suggested that in the chicken, the liver and the pancreas process vitamin K, and in particular menaquinone-4, in different ways. The observation of a 10-fold greater proportion of menaquinone-4 epoxide in the chicken pancreas as compared to the liver suggested that the vitamin K 2,3-epoxide reductase complex (VKOR) may not be as efficient in the avian pancreas as it is in the liver.

The production demands on laying hens also puts considerable strains on the bird's skeleton, which has also been shown to benefit from additional vitamin K3 in the diet [28]. From the discussion on the need to modify vitamin K3 into an active form of vitamin K, there is a strong likelihood of commercial avian health needs being met by the conversion of vitamin K3 into vitamin K2, as either menaquinone-4 or, with the potential for coprophagy, longer chain menaquinones from fecal bacteria.

Non-commercial avian species have received little attention for their vitamin K requirements. However, it should be noted that wild birds can often be the casualties of deliberate or accidental poisoning from anticoagulant agents that inhibit the vitamin K cycle [29, 30].

## **2.1. Rodentia and Lagomorpha**

### *2.1.1. The rat*

The wild the rat, as an omnivore, will derive their vitamin K from a variety of sources such as plant material and dead animal tissues and also from bacteria in decaying plant and animal materials. In the laboratory, the rat has been the most extensively investigated animal for vitamin K physiological and pathophysiological requirements. It has provided the standard, from which much human investigation has been initiated.

One of the fundamental elements of undertaking a rodent experiment on vitamin K has been the need to derive an animal with a vitamin K deficiency that survives long enough for investigation. Outside the use of a vitamin K-specific anticoagulants, such as warfarin and brodifacoum, this has been difficult to achieve nutritionally derived vitamin K deficiency [31,32]; Dr

Cees Vermeer, personal communication. With fastidious experimentation, it is possible to reduce vitamin K tissue levels in rats, and with certain diets, this can affect the intestinal vitamin K2-producing flora [33].

The reported high fecal coprophagy in the rat demonstrates that inducing vitamin K deficiency is difficult, even when animals are contained in elevated, wire-bottom metabolic cages with additional tail-cups on the rats to collect feces [34].

There is also tissue variation in the activity of VKOR in the rat [35], which can have implications, in some circumstances, on the functional efficacy of carboxylation of vitamin K-dependent proteins in extrahepatic tissues.

Polymorphism within the gene for VKOR has been associated with increased rodent resistance to anticoagulant rodenticides. This has profound potential commercial implications as the initial shift to more potent agents from the original warfarin used to control rodents has shown potential problems, due to environmental persistence, while the need to control rodents remains necessary [36].

### *2.1.2. The mouse*

In the wild, the mouse has the same exposure to sources of vitamin K as described for the rat, and, like the rat, mice in the wild are also showing increasing vitamin K anticoagulant resistance.

The mouse has been widely used as a laboratory model for many diseases due to a fast breeding rate and the potential for genetic homogeneity. The additional development of creating transgenic mice that over- or under-express a specific gene (or genes) together with site-directed, or conditional, gene manipulation has increased their importance in medical and scientific research enormously. These technologies have been exploited to demonstrate the essential nature of the VKOR enzyme [37].

The enzyme UbiA prenyltransferase domain-containing protein 1 (UBIAD1) is expressed across vertebrates and has been shown to synthesize the vitamin K2 congener menaquinone-4 [38]. Deletion of this gene in mice has been found to be lethal, preventing development beyond embryonic day 7.5 [39], suggesting a fundamentally important role for vitamin K2 in embryonic development.

Mice have the same limitations for vitamin K research as described for the rat. Inducing nutritional deficiency and preventing coprophagy is difficult, but can be achieved with some success using strict practices.

### *2.1.3. The rabbit*

Rabbits and hares are prolific herbivores, and therefore, a large amount of their vitamin K requirement is derived from vitamin K1. Although, as with rodents, rabbits consume substantial fecal matter; as long ago as 1882 it was reported that rabbits produce two types of fecal matter, one more liquid than the other [40]. Subsequently, the 'soft' feces were found to have a similar protein, fiber and nutrient composition to the caecal contents [41] and that coproph-

agy is also normal for the wild rabbit [42]. The caecum of the rabbit is a perfect environment for the incubation of numerous species of bacteria [43] and that the nursing doe probably carries the responsibility for ensuring her kittens have the appropriate intestinal/caecal microbial flora established early in life [44]. Indeed, an incorrect microbiota platform may lead to pathological conditions in the rabbit [45].

The sequelae from the discussion on coprophagy in the rabbit are that the recycling of bacterial contents from feces and maybe, importantly, the caecal content microbiota could contribute significantly to the animal's vitamin K status, in addition to their nutritional vitamin K1 intake. This has not been directly measured in the rabbit, but while there is ongoing debate about the vitamin K2 nutritional benefits from large bowel absorption of these fat-soluble vitamins, the small intestine absorption of vitamin is known [46].

There is no information on the hare that describes how it meets its full requirements for vitamin K, and presumably, the rabbit serves as an appropriate example to draw similar conclusions.

## 2.2. Agricultural animals

Agricultural species are important for essential food and other product production, and the health and well-being of livestock is important for their growth and reproduction. We will consider several major animal species, but with centuries of breeding and a wide diversity in breeds, we take a generic position in descriptions, except where specific health problems have been identified. The horse is included in this discussion as, while there is a substantial element of meat production from the horse and related species, it also has value in equine sport activities and a rapid growth rate is a selected feature for many of these activities.

### 2.2.1. *Ovine vitamin K requirements*

With over 200 sheep breeds that have been reared over thousands of years for different features, such as wool, milk, skins, meat and even the ability to clear vegetation, a great deal of genetic diversity has been engineered into the different flocks. As committed grazing herbivores, most of their vitamin K needs will be met by vitamin K1. However as a ruminant, there will be an added contribution through vitamin K2 from the microflora that undertake additional fermentation in their rumen. There is more direct published literature on vitamin K in the cow and the relationship to rumination, this is considered in Section 2.3.4.

Grazing can bring sheep into contact with toxic plants, such as giant fennel (*Ferula communis*) which contains a 4-hydroxycoumarin compound, a relative of the widely used human anticoagulant pharmaceutical and rodenticide warfarin. The giant fennel is widely distributed around the Mediterranean basin and has been associated with a hemorrhagic syndrome in several species of livestock, including sheep and goats [47]. In vivo experiments in sheep show that extracts from the plant can cause a range of symptoms, including hemorrhage [48, 49]. The active coumarol agents inhibit the VKOR enzyme in much the same way as warfarin, and there is a report of species susceptibility differences [50]. This syndrome can be successfully treated with vitamin K [51].

The new-born lamb, such as human neonates, can to some extent experience postpartum hemorrhage. In the case of the Rambouillet breed of sheep, there is a potentiation of this problem [52]. This problem has been causally linked to a genetic defect in the vitamin K-dependent carboxylase enzyme [53, 54].

### 2.2.2. *Caprine vitamin K requirements*

As with sheep, the goat has a long history of domestication by man and there are now many breeds of goat that have been generated, as with sheep, for a diverse range of reasons. In contrast to sheep, goats are browsers and will also crop tree branches and shrubs. While the goat is credited with eating anything, this is not the case, being inquisitive animals they test many things with their lips and mouth, and may then ingest inedible objects. The goat has the same chance of eating toxic plants as sheep, such as giant fennel.

The absence of the literature on hemorrhagic disease suggests that millennia of breeding programmes have not drawn in a vitamin K-related genetic hemorrhagic disease or, if it did, the mutation was lethal before the animal reached reproductive maturity.

### 2.2.3. *Porcine vitamin K requirements*

The history of the pig is even older than that of sheep and goats with indications that the pig was first domesticated 9–10,000 years ago in the Middle East [55]. Despite this heritage, there are many fewer breeds of pig than there are for sheep and goats. The pig, wild boar and hogs are natural scavengers and will consume anything edible, including decaying matter and fecal waste; the latter ability has been utilized by humans as part of local sewage management in the form of the 'pig-privy' [56]. This suggests that in the wild and in free-living domestic pig populations, long-chain vitamin K2 will be at least as important, if not more important, as vitamin K1 in maintaining the vitamin K status of the pig.

The vitamin K status of boar and hogs has not been the subject of investigation. However, husbandry of the domestic pig, due to its economic importance, has been considered extensively.

A comparative investigation into the coagulation status of several animal species found that the pig most closely mirrored the human neonate [57]. Therefore, the description of postpartum hemorrhage in the piglet due to vitamin K deficiency [58] is not too surprising as it is seen in the new-born human infant, where the recommendation is for vitamin K prophylaxis at birth. Increased hygienic conditions for farrowing sows housed in elevated sties preventing coprophagy and antibiotic usage were identified as potential factors in vitamin K deficiency in the pig. Around this time, supplementation with 2.2 mg/Kg feed with vitamin K3 was suggested for pigs if a vitamin K deficiency was suspected [59].

### 2.2.4. *Bovine vitamin K requirements*

Bovine species have as long an association with man as other domesticated animals. Cave paintings demonstrate that ancient man was aware of the auroch in his environment and was

probably a major predator of these animals. Domestication from the auroch has been suggested to date back to around 10,000 years in Asia and in the Near East. The modern-day descendants are the Zebu cattle in Asia and the common taurine cattle breeds in Europe. Genetic diversity from the DNA of skeletal remains of the European ancestors suggests that their diversity is so limited as to indicate that the modern taurine bovid has been derived from as few as 80 original cows [60].

The bovids, as ruminants, are as likely to derive their vitamin K requirements from rumen microflora as they are from plant sources. This has support when bovine liver menaquinone stores are considered against equine liver stores. The long-chain vitamin K2 congeners in bovine liver contain measurable menaquinones, up to menaquinone-13, while equine liver does not and vitamin K1 from plants is the principle form of vitamin K found in the liver of the horse [61–63].

A recent study looking at muscle levels of vitamin K in two bovine breeds, the Norwegian Red and the Jersey, raised under identical conditions in one location [64], found that the tissue distribution in muscle is distinctly different to that reported for the liver and different between the two breeds. This study found that there were two dominant forms of vitamin K, vitamin K1 and menaquinone-4. The former correlating with the muscle fat content, there being no equivalent relationship for menaquinone-4. These findings mirror a previous food screen report [65]. Also, different muscles in the two breeds found varying levels of vitamins K. The possibility of a relationship between the enzyme UBIAD1 and menaquinone-4 levels was not considered in the study, but this could, at least in part, explain some of the vitamin K2 findings.

Possibly, the single most relevant historical relationship between bovine species and vitamin K is the discovery of a vitamin K anticoagulant from the ingestion of spoilt sweet clover feed. This eventually led to the discovery and development of one of the world's most prescribed pharmaceutical drugs, warfarin [66–71].

#### *2.2.5. Equine vitamin K requirements*

The finding that horse liver contains predominantly vitamin K1 relates to the dietary source for this animal, but which also supports the contention that ruminants gain broader vitamin K support from bacterial vitamin K2 sources. In a large, rapidly growing animal like the horse, an adequate vitamin K supply may need to meet more than coagulation requirements. An indication of this may be suggested from a 4-week-old Standardbred colt initially presenting with vitamin K deficiency bleeding that continued to fail to thrive after normalization of the coagulation defect by the administration of vitamin K [72]. While there may have been other underlying pathologies, this observation of a vitamin K deficiency bleeding may have been the result of a genetic mutation in the breeding line as the stallion that sired the described foal had also previously sired a colt with coagulopathy health problems [72]. An inability to follow up the research prevented a definitive description of a vitamin K-dependent deficiency disease in these horses.

A study, with an interest in race horse growth, and in particular bone health [73], investigated the best form of vitamin K to administer orally to horses. Circulating vitamin K1 increased in



relation to the administered dose; however, menaquinone-4 administration did not show corresponding plasma level increases, the suggestion being that the horses in this study did not absorb this vitamin. Interestingly, administration of vitamin K3 did cause menaquinone-4 plasma levels to rise, which may relate to UBIAD1 synthesis of menaquinone-4 or intestinal flora.

Another consideration in equine health is for vitamin K3 administered parenterally; this form of intervention has been found to cause pronounced renal toxic effects [74, 75]. Reports of parenteral administration of vitamin K1 or K2 prophylaxis, or remedial coagulopathy, interventions were not obvious from screening the literature.

### 3. Piscine vitamin K requirements

The world growth in aquaculture has increased enormously over the last decade. From 2005 to 2013, the global worth of this industry has more than doubled to over 150 billion USD [76]. Any captive species will be dependent on being fed an appropriate diet for health in order to produce good quality product for an ever-demanding consumer population.

Fish, like other animals, are not able to *de novo* synthesize vitamin K and have to obtain it from their diet. Vitamin K deficiency in fish results in several familiar health problems that are found in terrestrial animals, including increased blood coagulation time, reduced growth, anemia, hemorrhage, weak bones, and occurrence of spinal curvature, short tails and increased mortality, together with problems specifically related to fish, such as loss of fin tissue [77–79].

The role of vitamin K in salmonids began to be investigated in the 1960s [80, 81], and today, minimum requirements for vitamin K supplement in fish feed are largely based on the effect of vitamin K on blood coagulation. Although estimates of dietary vitamin K requirement differ a great deal among fish species, and the quantitative requirement of vitamin K for most fish is still largely unknown. In addition, dietary studies on fish entail problems that are not encountered in terrestrial animals, such as vitamin leaching into their environment from supplemented feed [82].

Studies on zebrafish and Japanese puffer fish have found genes for vitamin K-dependent factors (VII, IX, X and prothrombin). Also, continuous exposure to warfarin causes spontaneous bleeding in zebrafish [83–85]. In common carp, warfarin prolonged the prothrombin time and activated partial thromboplastin time, whereas supplementation of menadione prevented increase in prothrombin time. For the large yellow croaker, the blood coagulation time generally decreased with increasing dietary menadione levels [86].

Menadione can be alkylated enzymatically to menaquinone-4 in tissues [87]. This conversion has been recorded in several fish species such as Atlantic salmon [88–90], Atlantic cod [91], cultured sardines [92], mummichog [93], ayu [94] and large yellow croaker [86].

The enzyme UBIAD1 that converts vitamin K1 into menaquinone-4 has been the subject of an investigation in a zebrafish mutant (*reddish*<sup>s587</sup>:*reh*). The fish develop normally for 24–36 h, but by 48 h they present with cranial hemorrhage [95]. The UBIAD1 gene was found to be

expressed as early as the single-cell stage. With continued development, the *reh* zebrafish mutant presents with higher expression of UBIAD1 in the vasculature than cardiac tissues and gene expression decreases with time. Introduction of an antisense morpholino oligonucleotide targeted splice to knockdown wild-type UBIAD1, or the administration of warfarin, produced similar vascular models to the *reh* mutant, with the warfarin challenge having notably less impact on cardiac tissue compared to the vascular effects. The defect in these fish could be salvaged by the introduction of wild-type zebrafish or human UBIAD1 mRNA, but not *reh* UBIAD1 mRNA. Similarly, the knockdown or warfarin-treated larvae could be rescued by the administration of vitamin K1 or menaquinone-4, vitamin K1 being used as the source of naphthoquinone for the UBIAD1 conversion to menaquinone-4. The overall finding that the UBIAD1 gene and enzyme expression has an important role in vascular endothelial cell survival has implications across all tissues and in cancer.

Bone and spinal deformities are a major problem in commercial fish farming. Deformities are not only an economic problem for fish farms, but also raise ethical and welfare issues for the aquaculture industry. The importance of vitamin K in fish skeletal health has increased interest in vitamin K requirement for normal bone development in fish. There are a few studies that have dealt specifically with the effect of vitamin K deficiency on fish bone health [78, 88, 90, 96, 97]. Tissue-specific gene expression of the vitamin K-dependent proteins, such as osteocalcin and matrix Gla protein (MGP), has been shown in the vertebrae of Atlantic salmon; however, dietary vitamin K was not found to regulate the expression of MGP [90, 98]. Interestingly, neither juvenile Atlantic salmon [90] or Atlantic salmon smolts [88] showed any sign of deformities on a diet lacking vitamin K supplementation.

Mummichog given feed that was not enriched with vitamin K grew thin, weak bones. Vitamin K deficiency induced bone structure abnormalities such as vertebral fusion and row irregularity, both in early development and during later growth [78, 96]. Furthermore, the offspring of vitamin K-deprived fish had higher incidences of abnormal vertebral formation 5 days after hatching when compared to larvae from fish fed a vitamin K-enriched diet [78]. In haddock, vitamin K appears to be necessary for bone mineralization [99]. However, vitamin K does not affect the number of osteoblast in haddock, while bone deformities coincided with an increased amount of osteoid and a decrease in bone mineral content. In the Senegalese sole given feed enriched with vitamin K, there was a notable improved larval growth performance and post-larval skeletal quality. Also, vitamin K modulated expression of protein involved in several biological processes including muscle contraction and development, cytoskeletal network, skin development, energy metabolism, protein chaperoning and folding, and bone development [97].

It now seems that vitamin K supply may be less than optimal for bone development, but sufficient to maintain normal growth and hemostasis [82, 87].

Fish feed is commonly enriched with vitamin K3 (menadione) in the form of water soluble salts, normally menadione sodium bisulphite (MSB) and menadione nicotinamide bisulphite (MNB) [82].

Using menadione in fish feed is, however, not without problems; too high a dosage, in particular MSB, has proven to cause reduced growth [91, 100]. Nevertheless, it remains one of the most common vitamin K supplements in fish feed.

## 4. Companion animals

The species and breeds within species that have become companion animals have expanded considerably. Rabbits, rats and various exotic animals are increasingly being kept as domestic pets. In this section, we focus on cats and dogs as they dominate the sector.

### 4.1. Canine vitamin K requirements

The dog has been domesticated for possibly even longer than agricultural animals [101–103]. The possible breeding matrix that has led to the huge array of current domestic dogs is known to carry several genetic defects [104], and while there are several well-described coagulopathies in the dog, vitamin K-specific deficiency is not a widely reported genetic mutation. One case study [105], considered that a black Labrador retriever admitted for a ovariohysterectomy later presented clinically with a vitamin K deficiency coagulopathy. After ruling out several other options, including fat malabsorption problems, ingested coumarin-based rodenticide, other xenobiotics, liver disease, and noting that the problem was resolved and managed by vitamin K administration, the authors suggested this was a possible case of vitamin K deficiency. This type of disease, therefore, remains a rare disease in the dog.

Conversely, scavenging for food has caused numerous cases of accidental coumarin-based anticoagulation poisonings in the dog that carry through all geographical regions and is repeatedly reported over time [106–108]. The problem has probably become exacerbated with the introduction of more persistent anticoagulant rodenticides.

Some early studies [109, 110] found that the vitamin K1 supplemented dog stored most of the administered tritiated vitamer in the liver. It was also noted that a proportion was converted into menaquinones, particularly menaquinone-4. In the light of the recent studies on UBIAD1 [38], this may have an explainable origin.

Incomplete gamma-carboxylation of vitamin K-dependent proteins has been implicated in human joint diseases [111, 112]. Some dog breeds have a predisposition to clinically significant arthritic diseases; however, studies on the potential to alter the course of arthritic disease in dogs with vitamin K have not been undertaken.

The commercial dog food manufacturers have an open policy on nutritional information in their products. Many do not explicitly refer to vitamin K content of their products. One major supplier notes that in their dry food product, vitamin K 'activity' is supplied as vitamin K3.

### 4.2. Feline vitamin K requirements

Cats are scavengers, they hunt birds and small animals including rodents, these activities bring them into contact with rodenticides [113], and therefore, it is not surprising that the principle

reason for veterinary use of vitamin K clinically is as a rescue medication due to accidental rodenticide intoxication. As with the dog, the problem has probably been exacerbated by the increasing use of the more persistent anticoagulants used as rodenticides that have replaced warfarin in order to overcome rodent warfarin resistance.

Starting in the 1950s in South West UK, a breed of Rex cat was developed out of some accidental breeding with feral tom cats, which led to some reverse mating into their own genetic line with the intent to maintain the Rex breed. One of these lines of development led to the Devon Rex cat. In 1990, three Devon Rex cats were described with a vitamin K deficiency character [114], after exclusion of other factors such as accidental anticoagulant ingestion, liver disease, intestinal malabsorption problems and treatment with vitamin K to correct their deficiency. The nature of the defect in the Devon Rex was investigated in the Netherlands, and this cat was found to have a decreased ability to gamma-carboxylate vitamin K-dependent clotting factors due to a decrease binding of reduced vitamin K and the clotting factors to the carboxylase enzyme [115].

With increasing age, cats also develop diseases that cause vitamin K deficiency coagulopathies, such as liver disease, inflammatory bowel disease and secondary malabsorption syndrome [116–118].

It is possible to induce a vitamin K deficiency through diet, presumably as the cat is not particularly associated with coprophagic behavior. In an early study of queens and their kittens fed either a commercial tuna- or a salmon-based fish diet, there was a notable increase in blood clotting times [119]. Where the information is available, current commercial cat diets provide vitamin K 'activity' in the form of vitamin K3, principally in the dry food products.

The cat is also prone to present clinically with chronic kidney disease (CKD) [120], and the reported prevalence is high, particularly in aged cats. It is interesting that the pathophysiology of feline CKD has been proposed to be sufficiently similar to human disease that the cat could provide a natural model to investigate human CKD [121]. In human CKD, there are several reports of an association with low vitamin K status [122] and a recent multi-ethnic study demonstrated an inverse association between estimated glomerular filtration rate and a functional marker of vitamin K deficiency, namely de-phospho-undercarboxylated matrix Gla protein [123].

Our pilot studies looking at circulating vitamin K in the healthy aged cats found that menaquinone-4 was the dominating form of vitamin K. This observation would suggest that the vitamin K3 in cat diet is converted through UBIAD1 to menaquinone-4, although this has not been specifically demonstrated. There is a line of thought that vitamin K may also be provided to the cat through colonic bacterial supply. The absence of long-chain menaquinones in our study suggest that this is unlikely and evidence supporting colonic absorption of fat-soluble vitamins K in general is limited [124].

Systemic inflammation is widely accepted as a dominant driver in the aetiology of CKD, and this is an active area of therapeutic interest [125]. The re-emerging observation of direct anti-inflammatory activity for vitamins K1 and K2 and in particular their common 7-carbon carboxylic acid catabolite [126–128] suggests that a low vitamin K status in CKD may also

translate to a weakened anti-inflammatory potential in the CKD patient and the CKD cat. The role of vitamin K in the homeostatic physiology of the kidney and the pathophysiology of feline CKD has not been the subject of focused study.

As with the ageing dog, cats are also likely to present with degenerative joint diseases [129] and the high co-morbidity relationship with CKD suggests that vitamin K deficiency in the ageing cat is possible [130]. The function of vitamin K in the aetiology of feline degenerative joint diseases remains to be investigated.

## 5. Concluding remarks

Vitamin K in animals as a general subject has not been systematically investigated. There are, however, common vitamin K-related health issues that animals share with man. These are predominantly associated with coagulopathies, but diseases of the musculoskeletal system and kidney injury may have considerable overlaps to the mutual benefit of man and animals.

The requirements for animal health and well-being are poorly defined for vitamin K, with, in some cases, misconceptions about the contributions to vitamin K status from colonic bacterial sources. Animals may have a distinctly greater reliance on vitamin K<sub>2</sub>, in large part due to their diet, which is regulated by the feed that is given to them. Furthermore, the upper alimentary canal supply of menaquinones may be of central benefit to support the vitamin K status of several species such as ruminants and possibly in particular rabbit kittens.

An exciting emerging area is the molecular regulation embryological development and growth by the *in situ* generation of the vitamin K<sub>2</sub> congener menaquinone-4, through the prenyltransferase UBIAD1. The work on the zebrafish has the potential to radiate across all species, and this may be of particular importance in conservation breeding programmes.

There is reason to suspect that in some metabolic and inflammatory diseases, there is a pronounced vitamin K deficiency. The prospect of intervening in some of these pathologies with vitamin K, such as kidney disease, is already being proposed for human patients. Defining the vitamin K requirements in different animals, beyond simple hepatic coagulation factor needs, may suggest newer approaches to veterinary medicine that could be investigated.

## Acknowledgements

This work was facilitated, in part, by the award of a senior fellowship to SJH by The Northern Norway Regional Health Authority.

## Author details

Jayde O'Neil<sup>1</sup>, Bethany Scarrott<sup>1</sup>, Ragnhild Aven Svalheim<sup>2</sup>, Jonathan Elliott<sup>1</sup> and Stephen J. Hodges<sup>1,3\*</sup>

\*Address all correspondence to: [shodges@rvc.ac.uk](mailto:shodges@rvc.ac.uk)

1 Department of Comparative Biomedical Sciences, The Royal Veterinary College, London, UK

2 Nofima AS, Muninbakken, Breivika, Tromsø, Norway

3 Anesthesia and Critical Care Research Group, The University Hospital of North Norway and UIT-The Arctic University of Norway, Tromsø, Norway

## References

- [1] Marcelli SW, Chang HT, Chapman T, Chalk PA, Miles RJ, Poole RK. The respiratory chain of *Helicobacter pylori*: identification of cytochromes and the effects of oxygen on cytochrome and menaquinone levels. *FEMS Microbiol Lett.* 1996;138(1):59–64.
- [2] Antunes A, Rainey FA, Wanner G, Taborda M, Pätzold J, Nobre MF, da Costa MS, Huber R. A new lineage of halophilic, wall-less, contractile bacteria from a brine-filled deep of the Red Sea. *J Bacteriol.* 2008;190(10):3580–7.
- [3] Srinivas TN, Anil Kumar P, Madhu S, Sunil B, Sharma TV, Shivaji S. *Cesiribacter andamanensis* gen. nov., sp. nov., isolated from a soil sample from a mud volcano. *Int J Syst Evol Microbiol.* 2011;61(7):1521–7. [Erratum: *Int J Syst Evol Microbiol.* 2011;61(10):2561]
- [4] Mimuro M, Tsuchiya T, Inoue H, Sakuragi Y, Itoh Y, Gotoh T, Miyashita H, Bryant DA, Kobayashi M. The secondary electron acceptor of photosystem I in *Gloeobacter violaceus* PCC 7421 is menaquinone-4 that is synthesized by a unique but unknown pathway. *FEBS Lett.* 2005;579(17):3493–6.
- [5] Taggart WV, Matschiner JT. Metabolism of menadione-6,7-3H in the rat. *Biochemistry.* 1969;8(3):1141–6.
- [6] Dialameh GH, Taggart WV, Matschiner JT, Olson RE. Isolation and characterization of menaquinone-4 as a product of menadione metabolism in chicks and rats. *Int J Vitam Nutr Res.* 1971;41(3):391–400.
- [7] Komai M, Shirakawa H, Kimura S. Newly developed model for vitamin K deficiency in germfree mice. *Int J Vitam Nutr Res.* 1988;58(1):55–9.
- [8] Thijssen HH, Drittij-Reijnders MJ. Vitamin K distribution in rat tissues: dietary phylloquinone is a source of tissue menaquinone-4. *Br J Nutr.* 1994;72(3):415–25.

- [9] Davidson RT, Foley AL, Engelke JA, Suttie JW. Conversion of dietary phylloquinone to tissue menaquinone-4 in rats is not dependent on gut bacteria. *J Nutr.* 1998;128(2):220–3.
- [10] Ronden JE, Drittij-Reijnders MJ, Vermeer C, Thijssen HH. Intestinal flora is not an intermediate in the phylloquinone-menaquinone-4 conversion in the rat. *Biochim Biophys Acta.* 1998;1379(1):69–75.
- [11] Buitenhuis HC, Soute BA, Vermeer C. Comparison of the vitamins K1, K2 and K3 as cofactors for the hepatic vitamin K-dependent carboxylase. *Biochim Biophys Acta.* 1990;1034(2):170–5.
- [12] Peterson WH, Peterson MS. Relation of bacteria to vitamins and other growth factors. *Bacteriol Rev.* 1945;9(2):49–109.
- [13] Glick MC, Zilliken F, György P. Supplementary growth promoting effect of 2-methyl-1,4-naphthoquinone of *Lactobacillus bifidus* var. *pennsylvanicus*. *J Bacteriol.* 1959;77(2):230–6.
- [14] Starr SE, Killgore GE, Dowell VR Jr. Comparison of Schaedler agar and trypticase soy-yeast extract agar for the cultivation of anaerobic bacteria. *Appl Microbiol.* 1971;22(4):655–8.
- [15] Bentley R, Meganathan R. Biosynthesis of vitamin K (menaquinone) in bacteria. *Microbiol Rev.* 1982;46(3):241–80.
- [16] Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol.* 1977;100(2):221–30.
- [17] Collins MD, Jones D. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev.* 1981;45(2):316–54.
- [18] Shah HN, Collins MD. Genus *Bacteroides*. A chemotaxonomical perspective. *J Appl Bacteriol.* 1983;55(3):403–16.
- [19] Dam H. Cholesterinsoffwechsel in Hühnereiern Hühnchen. *Biochem. Z* 1929;215:475–492.
- [20] Dam H. The antihæmorrhagic vitamin of the chick. *Biochem J.* 1935;29(6):1273–85.
- [21] Dam H, Schönheyder F, Lewis L. The requirement for vitamin K of some different species of animals. *Biochem J.* 1937;31(1):22–7.
- [22] Sullivan, T. and Al-Ubaidi YY. Linear growth and mineralization of bones in broad breasted bronze turkeys. *Poultry Sci.* 1963;42:46–49.
- [23] Sullivan, T. Skeletal problems in poultry: estimated annual cost and descriptions. *Poultry Sci.* 1994;73:879–882.
- [24] Fleming RH. Nutritional factors affecting poultry bone health. *Proc Nutr Soc.* 2008;67(2):177–83.

- [25] Fleming RH, McCormack HA, Mcteir L & Whitehead CC. Bone structure and strength at different ages in laying hens and effects of dietary particulate limestone, vitamin K and ascorbic acid. *Br Poult Sci* 1998;39, 434–440.
- [26] Zhang C, Li D, Wang F, Dong T. Effects of dietary vitamin K levels on bone quality in broilers. *Arch Tierernahr.* 2003;57(3):197–206.
- [27] Askim M, Haug A, Gadeholt G. Dietary intake of menaquinone-4 may determine hepatic and pancreatic menaquinone-4 in chickens. *Food Nutr Res.* 2012;56.
- [28] Fleming RH, McCormack HA, Mcteir L, Whitehead CC. Effects of dietary particulate limestone, vitamin K3 and fluoride and photostimulation on skeletal morphology and osteoporosis in laying hens. *Br Poult Sci* 2003;44:683–689.
- [29] Stone WB, Okoniewski JC, Stedelin JR. Poisoning of wildlife with anticoagulant rodenticides in New York. *J Wildl Dis.* 1999;35(2):187–93.
- [30] Guitart R, Sachana M, Caloni F, Croubels S, Vandenbroucke V, Berny P. Animal poisoning in Europe. Part 3: wildlife. *Vet J.* 2010;183(3):260–5.
- [31] Matschiner JT, Taggart WV. Bioassay of vitamin K by intracardial injection in deficient adult male rats. *J Nutr.* 1968;94(1):57–9.
- [32] Craciun AM, Groenen-van Dooren MM, Thijssen HH, Vermeer C. Induction of prothrombin synthesis by K-vitamins compared in vitamin K-deficient and in brodifacoum-treated rats. *Biochim Biophys Acta.* 1998;1380(1):75–81.
- [33] Mathers JC, Fernandez F, Hill MJ, McCarthy PT, Shearer MJ, Oxley A. Dietary modification of potential vitamin K supply from enteric bacterial menaquinones in rats. *Br J Nutr.* 1990;63(3):639–52.
- [34] Barnes RH and Fiala G. Effects of the prevention of coprophagy in the rat. I. Growth studies. *J Nutr.* 1958;64(4):533–40.
- [35] Hammed A, Matagrín B, Spohn G, Prouillac C, Benoit E, Lattard V. VKORC1L1, an enzyme rescuing the vitamin K 2,3-epoxide reductase activity in some extrahepatic tissues during anticoagulation therapy. *J Biol Chem.* 2013;288(40):28733–42.
- [36] Esther A, Endepols S, Freise J, Klemann N, Runge M, Pelz HJ. Rodenticide resistance and consequences. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* 2014;57(5):519–23.
- [37] Spohn G, Kleinridders A, Wunderlich FT, Watzka M, Zaucke F, Blumbach K, Geisen C, Seifried E, Müller C, Paulsson M, Brüning JC, Oldenburg J. VKORC1 deficiency in mice causes early postnatal lethality due to severe bleeding. *Thromb Haemost.* 2009;101(6):1044–50.
- [38] Nakagawa K, Hirota Y, Sawada N, Yuge N, Watanabe M, Uchino Y, Okuda N, Shimomura Y, Suhara Y, Okano T. Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme. *Nature.* 2010;468:117–121.



- [39] Nakagawa K, Sawada N, Hirota Y, Uchino Y, Suhara Y, Hasegawa T, Amizuka N, Okamoto T, Tsugawa N, Kamao M, Funahashi N, Okano T. Vitamin K2 biosynthetic enzyme, UBIAD1 is essential for embryonic development of mice. *PLoS One*. 2014;9(8):e104078.
- [40] Morot C. Des pelotes stomacal des leporides. *Mem. Soc. Centr. Med.Vet.* 1882;12, Ser. 1.
- [41] Eden A. Coprophagy in the rabbit: origin of the night feces. *Nature* 1940;145,628–629.
- [42] Southern HN. Coprophagy in the wild rabbit. *Nature* 1940;145,262.
- [43] Monteils V, Cauquil L, Combes S, Godon JJ, Gidenne T. Potential core species and satellite species in the bacterial community within the rabbit caecum. *FEMS Microbiol Ecol.* 2008;66(3):620–9.
- [44] Kovács M, Szendro Z, Milisits G, Bóta B, Bíró-Németh E, Radnai I, Pósa R, Bónai A, Kovács F, Horn P. Effect of nursing methods and faeces consumption on the development of the bacteroides, lactobacillus and coliform flora in the caecum of the newborn rabbits. *Reprod Nutr Dev.* 2006;46(2):205–10.
- [45] Bäuerl C, Collado MC, Zúñiga M, Blas E, Pérez Martínez G. Changes in cecal microbiota and mucosal gene expression revealed new aspects of epizootic rabbit enteropathy. *PLoS One*. 2014;9(8):e105707.
- [46] Hollander D, Rim E. Vitamin K2 absorption by rat everted small intestinal sacs. *Am J Physiol.* 1976;231(2):415–9.
- [47] Appendino G, Tagliapietra S, Gariboldi P, Nano GM, Picci V. Oxygenated prenylated coumarins from *F. communis*. *Phytochemistry* 1988;27:3619–3624.
- [48] Tligui N, Ruth GR. *Ferula communis* variety *brevifolia* intoxication of sheep. *Am J Vet Res.* 1994;55(11):1558–63.
- [49] Tligui N, Ruth GR, Felice LJ. Plasma ferulenol concentration and activity of clotting factors in sheep with *Ferula communis* variety *brevifolia* intoxication. *Am J Vet Res.* 1994;55(11):1564–1569.
- [50] Louvet MS, Gault G, Lefebvre S, Popowycz F, Boulven M, Besse S, Benoit E, Lattard V, Grancher D. Comparative inhibitory effect of prenylated coumarins, ferulenol and ferprenin, contained in the 'poisonous chemotype' of *Ferula communis* on mammal liver microsomal VKORC1 activity. *Phytochemistry.* 2015;118:124–30.
- [51] Lamnaouer D. Anticoagulant activity of coumarins from *Ferula communis* L. *Therapie.* 1999;54(6):747–51.
- [52] Baker DC, Robbe SL, Jacobson L, Manco-Johnson MJ, Holler L, Lefkowitz J. Hereditary deficiency of vitamin-K-dependent coagulation factors in Rambouillet sheep. *Blood Coagul Fibrinolysis.* 1999;10(2):75–80.

- [53] Johnson JS, Laegreid WS, Basaraba RJ, Baker DC. Truncated gamma-glut amyl carboxylase in rambouillet sheep. *Vet Pathol.* 2006;43(4):430–7.
- [54] Johnson JS, Soute BA, Olver CS, Baker DC. Defective gamma-glutamyl carboxylase activity and bleeding in Rambouillet sheep. *Vet Pathol.* 2006;43(5):726–32.
- [55] Rosenberg M and Redding RW. “Ancestral Pigs: A New (Guinea) Model for Pig Domestication in the Near East”. In: *Ancestors for the Pigs: Pigs in Prehistory*. S.M. Nelson ed., pp. 65–76. Masca Research Papers in Science and Archaeology Vol. 15. University of Pennsylvania Museum.
- [56] Nemeth D. “Privy-Pigs Prehistory? A Korean Analogy for Neolithic Chinese Substance Practices” In: *Ancestors for the Pigs: Pigs in Prehistory*. S.M. Nelson ed., pp. 11–25.
- [57] Massicotte P, Mitchell L, Andrew M. A comparative study of coagulation systems in newborn animals. *Pediatr Res.* 1986;20(10):961–5.
- [58] Schendel HE, Johnson BC. Vitamin K deficiency in the baby pig. *J. Nutr.* 1962 76:124–130.
- [59] Bowland JP Progress in swine nutrition for the veterinarian. *Can Vet J.* 1973;14(1):3–11.
- [60] Bollongino R, Burger J, Powell A, Mashkour M, Vigne JD, Thomas MG. Modern taurine cattle descended from small number of near-eastern founders. *Mol Biol Evol.* 2012;29(9):2101–4.
- [61] Matschiner JT, Taggart WV, Amelotti JM. The vitamin K content of beef liver. Detection of a new form of vitamin K. *Biochemistry.* 1967;6(5):1243–8.
- [62] Matschiner JT, Amelotti JM. Characterization of vitamin K from bovine liver. *J Lipid Res.* 1968;9(2):176–9.
- [63] Duello TJ, Matschiner JT. Identification of phylloquinone in horse liver. *Arch Biochem Biophys.* 1970;138(2):640–5.
- [64] Rødbotten R, Gundersen T, Vermeer C, Kirkhus B. Vitamin K2 in different bovine muscles and breeds. *Meat Sci.* 2014;97(1):49–53.
- [65] Elder SJ, Haytowitz DB, Howe J, Peterson JW, Booth SL. Vitamin K contents of meat, dairy, and fast food in the U.S. diet. *J Agric Food Chem.* 2006;54(2):463–7.
- [66] Campbell HA, and Link KP. Studies on the Hemorrhagic Sweet Clover Disease. IV. The isolation and crystallization of the hemorrhagic agent. *J. Biol. Chem.* 1941;138, 21–33.
- [67] Overman RS, Stahmann MA, Sullivan WR, Huebner CF, Campbell HA, Link P. Studies on the hemorrhagic sweet clover disease. VII. The effect of 3,3'-methylenebis (4-hydroxycoumarin) on the prothrombin time of the plasma of various animals. *J. Biol. Chem.* 1942;142, p. 941.

- [68] Overman RS, Stahmann MA, Huebner CF, Sullivan WR, Spero L, Doherty DG, Ikawa M, Graf L, Roseman S, Link KP. Anticoagulant activity and structure in the 4-hydroxycoumarin group. *J. Biol. Chem.* 1944;153:5–24.
- [69] Schofield FW. Damaged sweet clover: the cause of a new disease in cattle simulating hemorrhagic septicemia and blackleg. *J. Am. Vet. Med. Assoc.* 1924;64:553.
- [70] Roderick LMA. The pathology of sweet clover disease in cattle. *J. Am. Vet. Med. Assoc.* 1929;74:314.
- [71] Stahmann, MA, Huebner, CF, Link KP. Studies on the hemorrhagic sweet clover disease. XIII. *J. Biol. Chem.* 1941;138:513–527.
- [72] McGorum BC, Henderson IS, Stirling D, Wallace R, Haggart C, Thomas AE. Vitamin K deficiency bleeding in a Standardbred colt. *J Vet Intern Med.* 2009;23(6):1307–10.
- [73] Terachi T, Inoue Y, Ashihara N, Kobayashi M, Ando K, Matsui T. Plasma vitamin K concentration in horses supplemented with several vitamin K homologs. *J Anim Sci.* 2011;89(4):1056–61.
- [74] Rebhun WC, Tennant BC, Dill SG, King JM. Vitamin K3-induced renal toxicosis in the horse. *J Am Vet Med Assoc.* 1984;184(10):1237–9.
- [75] Maxie G, van Dreumel T, McMaster D, Baird J. Ontario. Menadione (vitamin K(3)) toxicity in six horses. *Can Vet J.* 1992;33(11):756–7.
- [76] <ftp://ftp.fao.org/FI/STAT/summary/a-6.pdf>
- [77] Taveekijakarn P, Miyazaki T, Matsumoto M, Arai S. Studies on vitamin K deficiency in amago salmon, *Oncorhynchus rhodurus* (Jordan & McGregor). *J Fish Dis* 1996;19:209–214.
- [78] Udagawa M. The Effect of parental vitamin K deficiency on bone structure in mummichog *Fundulus heteroclitus*. *J World Aquacult Soc* 2004;35:366–371.
- [79] Lall SP, Lewis-McCrea, LM. Role of nutrients in skeletal metabolism and pathology in fish—an overview. *Aquaculture* 2007;267:3–19.
- [80] Doolittle RF, Surgenor DM. Blood coagulation in fish. *Am J Physiol* 1962;203:964–970.
- [81] Poston HA. Effect of dietary vitamin K and sulfaguanidine on blood coagulation time, microhematocrit, and growth of immature brook trout. *Progr Fish-Cult* 1964;26:59–64.
- [82] Krossøy C, Waagbø R, Ørnsrud R. Vitamin K in fish nutrition. *Aquaculture Nutrition* 2011;17:585–594.
- [83] Jagadeeswaran P, Sheehan JP. Analysis of blood coagulation in the zebrafish. *Blood Cells, Molecules, and Diseases* 1999;25:239–249.
- [84] Jagadeeswaran P, Gregory M, Johnson S, Thankavel B. Haemostatic screening and identification of zebrafish mutants with coagulation pathway defects: an approach to identifying novel haemostatic genes in man. *Br J Haematol* 2000;110:946–956.

- [85] Hanumanthaiah R, Thankavel B, Day K, Gregory M, Jagadeeswaran P. Developmental expression of vitamin K-dependent gamma-carboxylase activity in zebrafish embryos: effect of warfarin. *Blood Cell Mol Dis* 2001;27:992–999.
- [86] Cheng L, Zhang W, Lin S, Xu W, Mai K. Effects of dietary vitamin K on growth performances, blood coagulation time and menaquinone-4 (MK-4) concentration in tissues of juvenile large yellow croaker *Pseudosciaena crocea*. *Aquacult Res*. 2015;46:1269–1275.
- [87] Udagawa M. Physiological role of vitamin K in fish. *Japan Agric Res Quart* 2000;34:279–284.
- [88] Graff IE, Waagbø R, Fivelstad S, Vermeer C, Lie Ø, Lundebye AK. A multivariate study on the effects of dietary vitamin K, vitamin D3 and calcium, and dissolved carbon dioxide on growth, bone minerals, vitamin status and health performance in smolting Atlantic salmon *Salmo salar* L. *J Fish Dis* 2002;25:599–614.
- [89] Graff IE, Krossøy C, Gjerdevik K, Julshamn K. Influence of dietary menadione nicotinamide bisulphite (vitamin K3) and phylloquinone (vitamin K1) on Atlantic salmon (*Salmo salar* L.) tissue levels, determined by high-performance liquid chromatography with fluorescence detection. *Aquacult Nutr* 2010;16:637–647.
- [90] Krossøy C, Waagbø R, Fjelldal PG, Wargelius A, Lock E-J, Graff IE, Ørnstrud R. Dietary menadione nicotinamide bisulphite (vitamin K3) does not affect growth or bone health in first-feeding fry of Atlantic salmon (*Salmo salar* L.). *Aquacult Nutr* 2009;15:638–649.
- [91] Grahl-Madsen E, Lie Ø. Effects of different levels of vitamin K in diets for cod (*Gadus morhua*). *Aquaculture* 1997;151:269–274.
- [92] Udagawa M, Nakazoe J-i, Murai T. Tissue distribution of phylloquinone and menaquinone-4 in sardine, *Sardinops Melanostictus*. *Comp Biochem Physiol Part B: Comp Biochem*. 1993;106:297–301.
- [93] Udagawa M, Hirose K. Physiological abnormality and the tissue vitamin K level of mummichog *Fundulus heteroclitus* fed with a vitamin K free diet. *Fisheries Sci*. 1998;64:612–616.
- [94] Udagawa M, Nakazoe J-I, Murai T. Comparison of the composition of vitamin K in different forms between cultured and wild ayu (*Plecoglossus altivelis*). *Fisheries Sci*. 1993;65:331–332.
- [95] Hegarty JM, Yang H, Chi NC. UBIAD1-mediated vitamin K2 synthesis is required for vascular endothelial cell survival and development. *Development*. 2013;140(8):1713–9.
- [96] Udagawa M. The effect of dietary vitamin K (phylloquinone and menadione) levels on the vertebral formation in mummichog *Fundulus heteroclitus*. *Fisheries Sci*. 2001;67:104–109.
- [97] Richard N, Fernández I, Wulff T, Hamre K, Cancela L, Conceição LE, Gavaia PJ. Dietary supplementation with vitamin K affects transcriptome and proteome of Senegalese

- sole, improving larval performance and quality. *Marine biotechnology* (New York, N.Y.) 2014;16:522–537.
- [98] Krossøy C, Ørnsrud R, Wargelius A. Differential gene expression of *bgp* and *mgp* in trabecular and compact bone of Atlantic salmon (*Salmo salar* L.) vertebrae. *J Anatomy* 2009;215:663–672.
- [99] Roy PK, Lall, SP. Vitamin K deficiency inhibits mineralization and enhances deformity in vertebrae of haddock (*Melanogrammus aeglefinus* L.). *Comp Biochem Physiol Part B, Biochem Mol Biol* 2007;148:174–183.
- [100] Grisdale-Helland B, Helland SJ, Åsgård T. Problems associated with the present use of menadione sodium bisulfite and vitamin A in diets for Atlantic salmon. *Aquaculture* 1991;92:351–358.
- [101] Freedman AH, Gronau I, Schweizer RM, Ortega-Del Vecchyo D, Han E, Silva PM, Galaverni M, Fan Z, Marx P, Lorente-Galdos B, Beale H, Ramirez O, Hormozdiari F, Alkan C, Vilà C, Squire K, Geffen E, Kusak J, Boyko AR, Parker HG, Lee C, Tadisotla V, Wilton A, Siepel A, Bustamante CD, Harkins TT, Nelson SF, Ostrander EA, Marques-Bonet T, Wayne RK, Novembre J. Genome sequencing highlights the dynamic early history of dogs. *PLoS Genet.* 2014(1):e1004016.
- [102] Duleba A, Skonieczna K, Bogdanowicz W, Malyarchuk B, Grzybowski T. Complete mitochondrial genome database and standardized classification system for *Canis lupus familiaris*. *Forensic Sci Intl: Genetics* 2015;19:123–129.
- [103] Skoglund P, Ersmark E, Palkopoulou E, Dalén L. Ancient wolf genome reveals an early divergence of domestic dog ancestors and admixture into high-latitude breeds. *Curr Biol.* 2015;25(11):1515–9.
- [104] Mellersh C. DNA testing and domestic dogs. *Mamm Genome.* 2012;23(1–2):109–23.
- [105] Mason DJ, Abrams-Ogg A, Allen D, Gentry PA, Gadd KR. Vitamin K-dependent coagulopathy in a black Labrador Retriever. *J Vet Intern Med.* 2002;16(4):485–8.
- [106] Mount ME, Kass PH. Diagnostic importance of vitamin K1 and its epoxide measured in serum of dogs exposed to an anticoagulant rodenticide. *Am J Vet Res.* 1989;50(10):1704–9.
- [107] Robben JH, Mout HC, Kuijpers EA. Anticoagulant rodenticide poisoning in dogs in The Netherlands. *Tijdschr Diergeneeskd.* 1997;122(17):466–71.
- [108] Waddell LS, Poppenga RH, Drobatz KJ. Anticoagulant rodenticide screening in dogs: 123 cases (1996–2003). *J Am Vet Med Assoc.* 2013;242(4):516–21.
- [109] Duello TJ, Matschiner JT. Characterization of vitamin K from pig liver and dog liver. *Arch Biochem Biophys.* 1971 May;144(1):330–8.
- [110] Duello TJ, Matschiner JT. The relationship between the storage forms of vitamin K and dietary phyloquinone in the dog. *Int J Vitam Nutr Res.* 1971;41(2):180–8.

- [111] Hamidi MS, Cheung AM. Vitamin K and musculoskeletal health in postmenopausal women. *Mol Nutr Food Res*. 2014;58(8):1647–57.
- [112] Shea MK, Kritchevsky SB, Hsu FC, Nevitt M, Booth SL, Kwok CK, McAlindon TE, Vermeer C, Drummen N, Harris TB, Womack C, Loeser RF. Health ABC Study. The association between vitamin K status and knee osteoarthritis features in older adults: the Health, Aging and Body Composition Study. *Osteoarthritis Cartilage*. 2015;23(3):370–8.
- [113] Kohn B, Weingart C, Giger U. Haemorrhage in seven cats with suspected anticoagulant rodenticide intoxication. *J Feline Med Surg*. 2003;5(5):295–304.
- [114] Maddison JE, Watson AD, Eade IG, Exner T. Vitamin K-dependent multifactor coagulopathy in Devon Rex cats. *J Am Vet Med Assoc*. 1990;197(11):1495–7.
- [115] Soute BA, Ulrich MM, Watson AD, Maddison JE, Ebberink RH, Vermeer C. Congenital deficiency of all vitamin K-dependent blood coagulation factors due to a defective vitamin K-dependent carboxylase in Devon Rex cats. *Thromb Haemost*. 1992;68(5):521–5.
- [116] Edwards DF, Russell RG. Probable vitamin K-deficient bleeding in two cats with malabsorption syndrome secondary to lymphocytic-plasmacytic enteritis. *J Vet Intern Med*. 1987;1(3):97–101.
- [117] Lisciandro SC, Hohenhaus A, Brooks M. Coagulation abnormalities in 22 cats with naturally occurring liver disease. *J Vet Intern Med*. 1998;12(2):71–5.
- [118] Center SA, Warner K, Corbett J, Randolph JF, Erb HN. Proteins invoked by vitamin K absence and clotting times in clinically ill cats. *J Vet Intern Med*. 2000;14(3):292–7.
- [119] Strieker MJ, Morris JG, Feldman BF, Rogers QR. Vitamin K deficiency in cats fed commercial fish-based diets. *J Small Anim Pract*. 1996;37(7):322–6.
- [120] Brown CA, Elliott J, Schmiedt CW, Brown SA. Chronic kidney disease in aged cats: clinical features, morphology, and proposed pathogenesis. *Vet Pathol*. 2016;53(2):309–26.
- [121] Shipov A, Segev G, Meltzer H, Milrad M, Brenner O, Atkins A, Shahar R. The effect of naturally occurring chronic kidney disease on the micro-structural and mechanical properties of bone. *PLoS One*. 2014;9(10):e110057.
- [122] McCabe KM, Adams MA, Holden RM. Vitamin K status in chronic kidney disease. *Nutrients*. 2013;5(11):4390–8.
- [123] Wei FF, Drummen NE, Schutte AE, Thijs L, Jacobs L, Petit T, Yang WY, Smith W, Zhang ZY, Gu YM, Kuznetsova T, Verhamme P, Allegaert K, Schutte R, Lerut E, Evenepoel P, Vermeer C, Staessen JA. Vitamin K dependent protection of renal function in multi-ethnic population studies. *EBioMedicine*. 2016;4:162–9.
- [124] Shearer MJ. Vitamin K metabolism and nutriture. *Blood Rev*. 1992;6(2):92–104.

- [125] Machowska A, Carrero JJ, Lindholm B, Stenvinkel P. Therapeutics targeting persistent inflammation in chronic kidney disease. *Transl Res.* 2016;167(1):204–13.
- [126] Weiser H, Kormann A. Pharmacological properties of vitamin K1 and analogues: Antidote functions, analgesic and antiphlogistic properties. Unpublished results presented as a poster at the 17th Steenbock Symposium June 1987, Current Advances in Vitamin K Research, University of Wisconsin, Madison, USA. 1988.
- [127] Reddi K, Henderson B, Meghji S, Wilson M, Poole S, Hopper C, Harris M, Hodges SJ. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. *Cytokine.* 1995;7(3): 287–90.
- [128] Soper RJ, Oguz C, Emery R, Pitsillides AA, Hodges SJ. Vitamin K catabolite inhibition of ovariectomy-induced bone loss: structure-activity relationship considerations. *Mol Nutr Food Res.* 2014;58(8):1658–66.
- [129] Lascelles BD, Henry JB 3rd, Brown J, Robertson I, Sumrell AT, Simpson W, Wheeler S, Hansen BD, Zamprogno H, Freire M, Pease A. Cross-sectional study of the prevalence of radiographic degenerative joint disease in domesticated cats. *Vet Surg.* 2010;39(5): 535–44.
- [130] Marino CL, Lascelles BD, Vaden SL, Gruen ME, Marks SL. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. *J Feline Med Surg.* 2014;16(6):465–72.