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Physiological Role of Vitamin K in Fish — Review —

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Abstract

Basic information on the role of vitamin K in fish was described in relation to dietary intake. Vitamin K in the fish tissues was mainly concentrated in the liver and gonads, with a small amount in muscles. There was a difference in the concentration of vitamin K between demersal and pelagic fish depending on the habitat and between cultured fish and wild fish. The composition and content of vitamin K in the gastrointestinal tract which were very similar to those of the food consumed differed in the tissues, suggesting that all the vitamin K in food was not transferred to the tissues of fish. There was no clear indication that vitamin K deficiency affected the growth rate or mortality in fish, although it was reported that vitamin K deficiency caused anemia, prolonged prothrombin time, histopathological changes and bone abnormalities. Therefore, more studies are required to determine the role of vitamin K in fish.

Discipline: Fisheries
Additional key words: menaquinone, phylloquinone, fish nutrition

Introduction

Vitamin K was first discovered by Dam[3] as a substance playing an important role in the synthesis of blood-clotting factors. Vitamin K is a group name for a series of related compounds that act as cofactor for the microsomal enzyme γ-glutamylcarboxylase. This enzyme is involved in the post-translational conversion of peptide-bound glutamate residues into γ-carboxyglutamate (Gla), which occurs in a number of blood coagulation factors and bone proteins[4,7,27]. Natural forms of vitamin K include phylloquinone (PK) and menaquinone (MK). PK designated as vitamin K_1 occurs abundantly in plant tissues while MK, vitamin K_2, is mainly produced by bacteria. Furthermore, a variety of diseases due to vitamin K deficiency has been reported in mammals. In particular, human infants, a few weeks old, are prone to hemorrhage due to vitamin K deficiency. In addition, the attention has since been increasingly focused on the importance of vitamin K for normal bone development. However, the role of vitamin K in fish has not been well-documented. In the following review, the distribution and absorption of vitamin K in relation to the intake of vitamin K in fish will be outlined.

Derivatives of vitamin K

There are several derivatives of vitamin K (Fig. 1) as follows: 1) PK called vitamin K_1, is produced by plants; 2) MK which is synthesized by microorganisms is called vitamin K_2 and has long side-chains arranged in MK-n series. MK-4 consists of short isoprene units and MK-9 is one of the typical long-chain MKs produced by bacteria; 3) Synthetic analogues of menadione (MD) designated as vitamin K_3 have no side-chains, and are utilized for animal and fish commercial diets. Water-soluble salt of synthetic menadione, menadione sodium bisulfite (MSB) is generally supplemented in the commercial fish diets. Parts of PK, MK and MD are metabolized to MK-4 in the tissues[13].

Vitamin K in fish

Tissue distribution of vitamin K in fish varies with the feed habitat, for example, demersal and pelagic fish[22] as well as between cultured and wild fish[21,23].

Food habits differ markedly between pelagic fish and demersal fish. Pelagic fish feed on plankton and small fish, while demersal fish feed chiefly on benthos. Benthos are the organisms which live in the benthic zone

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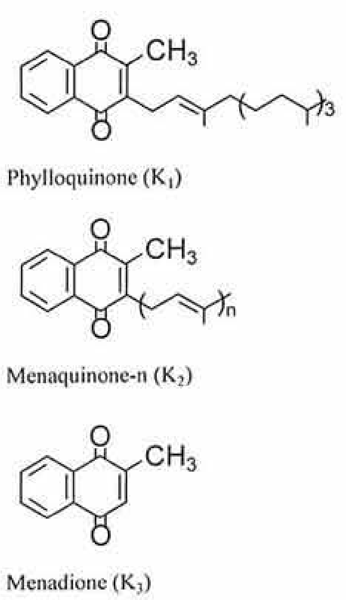

Fig. 1. Chemical structure of vitamin K

of the sea. The PK and MK contents in the pelagic and demersal fish tissues are illustrated in Fig. 2[22]. None of the samples showed even a trace of MK-9. In mackerel, MK-6 was detected unlike other long-chain MKs. The content of PK was much higher than that of MKs, particularly in the liver where the PK content was as high as 3–4 times that in the heart and kidney. However, only a trace amount of vitamin K was detected in the ordinary and dark muscle tissues. In striking contrast to mackerel, MK-4 appeared to be the major vitamin K in the heart and gonads of saury with a very low level of PK. Furthermore, a negligible amount of vitamin K was present in the liver. The total vitamin K content was, however, much lower than that in mackerel.

In the case of marbled sole, significant amounts of long-chain MKs (MK-6 and 7) were detected together with MK-4 in the heart and kidney. Furthermore, MK-8 was also found in the kidney. On the other hand, in sillago, PK and MK-4 were commonly distributed in various organs, whereas MK-6, 7 and 8 were found only in

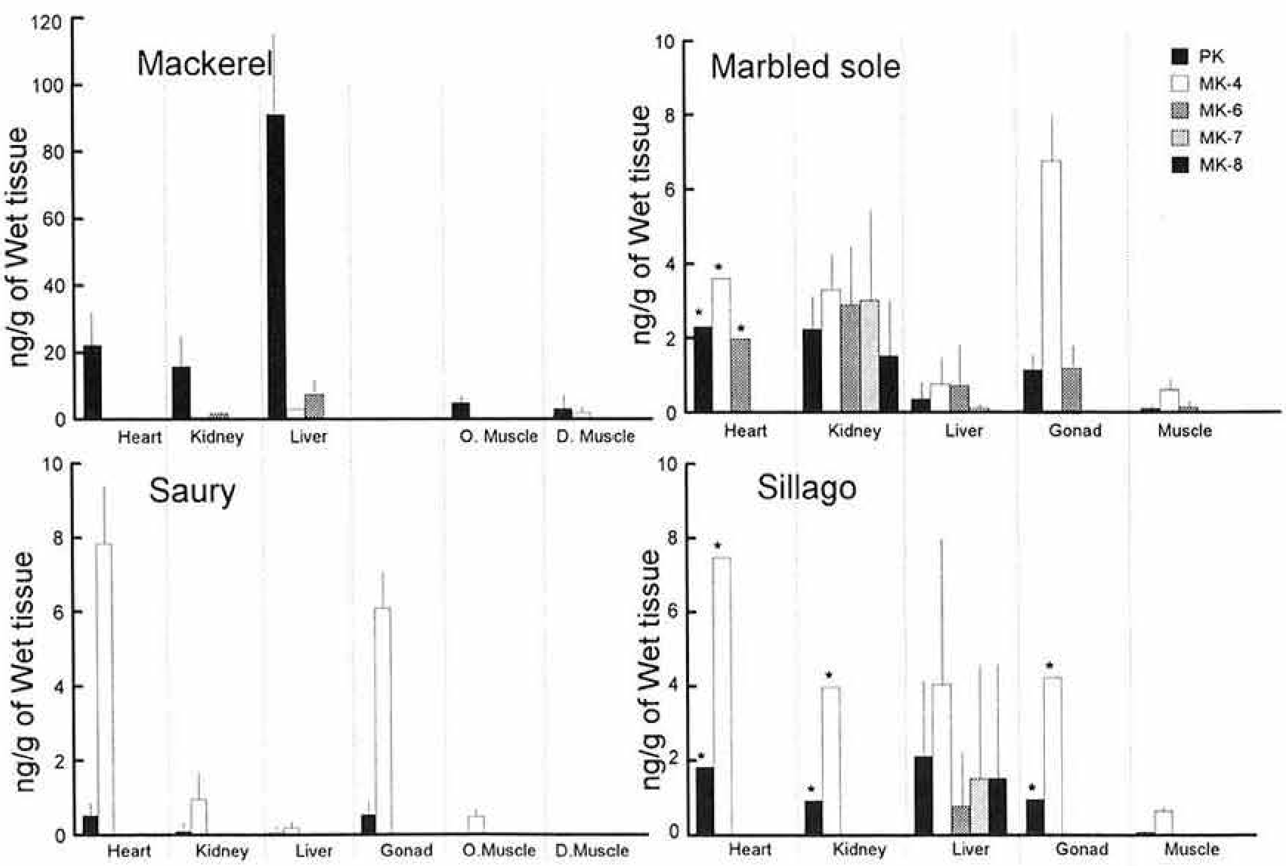

Fig. 2. Contents of phylloquinone(PK) and menaquinone(MK)-4, 6, 7, 8, 9 in various tissues of mackerel, saury, marbled sole and sillago

O. muscle: Ordinary muscle, D. muscle: Dark muscle. Values are means ± SEM of 5 fish.

* Indicates that the values were measured by pooling specimens from 5 fish.

MK-9 was also analyzed, but not detected in all the tissues examined[22].

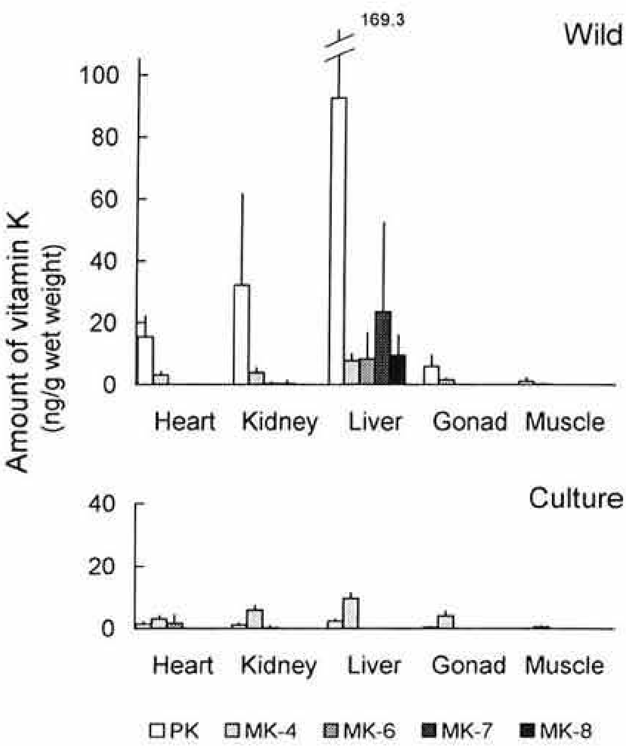

Fig. 3. Contents of phylloquinone and menaquinones in different tissues of wild and cultured ayu

Values are means ± SEM of 5 fish (cultured fish) and 10 fish (wild fish). MK-9 was not detected in all the tissues examined[23].

the liver. In both marbled sole and sillago, the PK level was higher than that of saury on the whole but very much lower than that of mackerel.

In an another report, it was stated that the PK contents in the serum, heart, kidney, gonads and liver of wild sardine were much higher than those of cultured sardine, especially in the liver[21]. MK-4 contents in cultured sardine were significantly higher than those of wild sardine in the serum, heart, kidney, gonads and liver.

In the tissues of wild ayu, PK appeared to be the major form of vitamin K, and MKs were present at very low levels (Fig. 3)[23]. Long-chain MKs in different forms were present in the liver, but their quantity was lower than that of PK. Only MK-6 was detected in tissues other than the liver. On the other hand, in the tissues of cultured ayu, the major form of vitamin K was MK-4, while MK-6 was detected only in the heart and kidney. Unlike in the wild ayu, no long-chain MKs were observed in the liver.

Contents of consumed feed

The human vitamin K requirement is assumed to be fulfilled by both dietary PK and bacterially produced MKs[8]. Vitamin K concentration in dietary intake by fish has been reported[21-23].

The level of PK in the plankton which is assumed to be consumed by wild sardine was almost 30 times higher than that of MK-4. In *Euphausia pacifica*, which is consumed by pelagic fish, the PK and MK levels were low. On the other hand, in *Polychaeta* spp., benthos which is consumed by demersal fish, MK-7 was the major vitamin K followed by MK-6 and MK-8, with a trace or negligible amount of PK.

GI tract contents

As shown in Table 1, the contents of PK and MKs in the gastrointestinal (GI) tract differed markedly among the species. In the GI tract of marbled sole and sillago, large amounts of long-chains MKs were detected. However, in the mackerel and saury, the contents of long-chains MKs were less than 1/100 of those of marbled sole and sillago. The GI tract of mackerel contained large amounts of PK, MK-6 and 7, and that of saury large amounts of MK-4, 6 and 7. However, in the GI tract of marbled sole and sillago, MK-6 and 7 were the major components and the proportions of PK and MK-4 were very low. The composition of vitamin K was very similar between the stomach and intestine of the same species of fish, and consumed feed composition of vitamin K was similar to that of the GI tract of mackerel and saury except for a low content of PK.

In the ayu, PK is abundant in diatoms. In the GI tract of wild ayu fed with diatoms, the PK content accounted for 60 – 80% of the total vitamin K content. It is, therefore, certain that the PK in the GI tract originated from diatoms in the diet. As for cultured ayu, long-chain MKs were the major form of vitamin K in the GI tract. Because MSB cannot be detected by the same HPLC method, MD could not be detected in the GI tract.

Absorption of MKs

In the GI tract, although MK-7 was one of the major vitamin K components in mackerel and sillago, MK-7 was not abundant in the other tissues of these fishes. In the case of mackerel and saury, the GI tract contained a large amount of long-chain MKs while in the tissues, only a small amount of long-chain MKs was detected. In each group of fish, large quantities of long-chain MKs, which were considered to be of bacterial origin, were detected in the GI tract (Table 1). However, only small amounts were found in the tissues (Fig. 2), suggesting that the absorption of long-chain MKs in the tissues of fish is negligible. Further studies are required to confirm the existence of MK-producing bacteria in the GI tract and the low utilization of their by-products. Based on this observation, it is likely that MK-producing bacteria could not be a major source of vitamin K in the ayu. These findings also suggest that the extent of absorption and/or conversion of vitamin K in the tissues of the ayu vary with the forms of vitamin K. The same results were obtained in the ayu, indicating that all the vitamin K derivatives in food were not transferred to the tissues of fish and that a selective absorption mechanism may be involved.

Tissue-specific accumulation of PK and MKs

In the liver of the mackerel and sardine, the PK contents were very high, unlike in the tissues of demersal fish or cultured fish. This observation is similar to that in the ayu where in the liver, most of the vitamin K derivatives present were in the form of PK in the wild ayu and MK-4 in the cultured ayu. The quantity of PK in the wild ayu was about 10 times higher than that of MK-4 in the cultured ayu, presumably due to the fact that vitamin K in different forms may be selectively absorbed and deposited in the tissues. Furthermore, the selection and degree of deposition may differ among the species of fish and/or organs. Though the contents of long-chain MKs in the tissues of fish were not as high as those in the GI tract, the kidney and liver contained various forms of long-chain MKs.

The absorption of vitamin K derivatives has not been fully elucidated. Kindberg and Suttie previously determined the rate of hepatic turnover and metabolism of phylloquinone in rats[9]. Birgit and Suttie[2] described the turnover of MK-9 in rat liver and compared it with that of PK. However, there are few reports in fish. Grahl-Madsen and Lie[5] reported that the level of MK-4 in the liver increased with the increase of the dietary MSB level in cod.

It was reported that the absorption of various vitamin K derivatives in fish tissues differed depending on the diets supplemented with such derivatives[25]. PK-rich diet led to the increase of the PK level in the fish tissues and plasma. On the other hand, diets rich in short and long-chain MKs did not result in high contents of MKs in the plasma, while the content slightly increased in the tissues. These results suggest that PK accumulation in the fish body is higher than that of MKs.

Deficiency in vitamin K

There are many reports on vitamin K requirements for animals except for fish, and vitamin K is generally considered to be an essential nutrient for animals. Woodward[29] gave quantitative estimates of dietary requirement of vitamin K for salmonids. Poston[15] reported that in the brook trout, the blood coagulation time was delayed and the microhematocrit value decreased by vitamin K deficiency, but that the growth rate was not appreciably affected. Kitamura et al.[10] also reported that in the rainbow trout, vitamin K deficiency was only associated with anemia. As for the catfish, no symptoms of vitamin K deficiency were observed[12]. On the other hand, Taveekijakarn et al.[20] reported that the mortality level in vitamin K-deficient fish amago salmon

Table 1. Contents of phylloquinone (PK) and menaquinones (MKs) in the gastrointestinal tract of fish[22]

(ng/g of wet substance)

	PK (%)	MK-4 (%)	MK-6(%)	MK-7 (%)	MK-8 (%)	MK-9 (%)
Mackerel						
Stomach	3.97±2.04(32)	1.29±0.26(10)	3.83±0.87(31)	3.28±2.31(27)	N.D.	N.D.
Intestine	2.01±0.98(34)	0.24±0.03 (4)	2.37±1.05(40)	1.07±0.24(18)	0.18±0.28 (3)	N.D.
Saury						
Stomach	0.74±0.65(10)	2.62±1.49(35)	2.11±2.04(28)	1.96±1.53(26)	N.D.	N.D.
Intestine	2.48±0.69(13)	5.79±1.03(29)	5.61±2.05(28)	5.98±4.23(30)	N.D.	N.D.
Marbled sole						
Stomach	54.8±50.8 (6)	20.3 ±15.1 (2)	228 ±343(24)	408±582 (44)	160 ±143(17)	62.7±67.0 (7)
Intestine	12.6±11.7 (4)	4.76± 2.72 (2)	95.1±164(30)	159±279 (49)	39.1± 54.0(12)	13.7±23.8 (4)
Sillago						
Stomach	9.82±11.8 (6)	6.34±3.66 (4)	67.5±103 (43)	53.5±66.7(34)	14.6±14.6 (9)	3.98± 5.97 (3)
Intestine	19.9 ±21.9 (8)	10.0 ±5.37 (4)	75.1± 89.0(28)	94.1±54.4(36)	47.2±48.4(18)	18.4 ±25.8 (7)

Values are means ±SEM of 5 fish.

(%): Percentage of each vitamin K to total vitamin K volume.

N.D.: not detected.

reached 50% and histopathological changes were detected.

It was also reported that fish during the spawning season was affected by a deficiency in vitamin K[24]. This report describes the experimental results of the changes in the vitamin K content in different organs of the mummichog, *Fundulus heteroclitus*, fed with a vitamin K-supplemented or deficient diet for 11 weeks. None of the male or female fish which were fed with a diet supplemented with MSB died. In contrast, most of the male fish died during the experiment, while female fish seldom died. Because a large number of immature erythrocytes were observed, it was assumed that in the female fish hematopenia and/or hematopoiesis had been affected by a deficiency in vitamin K. However, larval mortality was not affected by vitamin K deficiency. These results suggest that vitamin K intake is necessary for the mummichog, particularly the male fish during the spawning season even though the fish can continue to grow without any vitamin K supplement.

Effect on normal bone development

Biological function of vitamin K had been limited to the role of cofactor in blood coagulation. Recently, the important role of vitamin K in normal bone development has been revealed in mammals[28]. Residue-specific carboxylation of proteins is one of the functions of vitamin K and calcium-binding proteins such as osteocalcin and matrix-Gla protein also require vitamin K for their carboxylation. Thus, the importance of vitamin K in mammalian bone development has attracted the interest of many researchers[11,17], while the participation of vitamin K in fish bone development has not been investigated. The report described that diets without vitamin K caused an apparently higher incidence of bone abnormalities in the mummichog, *Fundulus heteroclitus*, than diets supplemented with MSB or phylloquinone[26]. These results clearly indicate that vitamin K is necessary for normal bone development in the mummichog as in the case of mammals.

Conclusion

The quantity of vitamin K required in fish is still unknown. There has been a growing interest in the possible involvement of higher intake of vitamin K in normal bone development in mammals[1,17,28] as well as in fish. It has been recognized that vitamin K supply may be suboptimal for bone but sufficient to maintain normal growth or reduce mortality. However, it has been reported that menadione and its analogues are toxic to certain animals (mice, rats, horses and humans) and cause abnormalities in the liver, kidneys and lungs, as well as hemorrhage, hemolytic anemia and other physiological abnormalities[14,16,18,19]. Grisdale[6] reported that the use of MSB in salmonid diets may not be optimal in relation to fish growth and health. The low availability of MK from bacterial origin or MSB compared with that of PK will have to be examined carefully if possible levels of supplementation are considered.

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