Chapter 12
Vitamin nutrition

C. Lauridsen & S. K. Jensen
E-mail: charlotte.lauridsen@agrsci.dk

This chapter deals with:

- A basic understanding of functions of and requirements for vitamins in nutrition of pigs.
- Knowledge of vitamin absorption, deficiency and safety.
- A clear understanding of the role of vitamins in reproduction, growth, health and immunity.
- Insight into factors in production practices influencing vitamin nutrition of pigs.

1. Introduction

The term “vitamin” (“vital amine”) was coined by the Polish biochemist Casimir Funk 100 years ago, and the discovery of vitamins was concentrated mainly in the first half of the 20th century. During that time, the focus of both animal and human nutrition research was on symptoms of vitamin deficiency and hence associated diseases. In animal nutrition, estimation of vitamin requirements is still often based on levels required to prevent deficiency rather than the levels required for optimal production performance. Little research focus has been given to optimisation in relation to other physiological responses, although most of the vitamins play an important role in metabolism in ensuring health, reproduction, immunity, and well-being. The high productivity level characterizing today’s intensive pig farming management calls for optimal levels to maintain growth, gestation, and lactation. In other farming systems, such as outdoor or organic production systems, additional challenges for the application of vitamins are in focus. The purpose of the present chapter is to provide the reader with basic knowledge regarding vitamins in pig nutrition, and to combine this information with the recent scientific advances in vitamin nutrition of pigs.

2. Definition and requirement of vitamins

Vitamins are organic compounds required in the diet in only small amounts. The thirteen existing substances considered as vitamins according to this definition are generally divided into two main groups: fat-soluble vitamins and water-soluble vitamins. This classification is valuable as it indicates whether the vitamin is likely to be absorbed similarly to either lipids or to water-soluble nutrients. Fat absorption mechanisms are described in Chapter 10, and for water-soluble nutrients the interested reader should look into some more biochemical oriented books, because in several cases these vitamins act as co-factors and transport proteins involved in the digestion process. Fat-soluble vitamins include vitamins A, D, E, and K, and they may be stored in the body when dietary provision is above the animal's requirement. Water-soluble vitamins include thiamine (B_1), riboflavin (B_2), niacin (B_3), panthothenic acid (B_5), pyridoxine (B_6), biotin (B_7), and cyanocobalamin (B_{12}). However, in terms of human and other primates vitamin C is also considered a vitamin due to lack of the enzyme L-gulonolactone oxidase (GULO), which is required in the last step of vitamin C synthesis due to a defective form of the gene for this enzyme. Pigs and most other animals have this enzyme and are capable of synthesizing vitamin C from glucose. Vitamin C is therefore not considered a vitamin for pigs and is thus not routinely added to pig feed. Among other vitamin-like compounds essential fatty acids (also named "vitamin F"), linoleic and linolenic acids should be mentioned, because they are required in the diet in only small amounts, but, as for vitamin C, no dietary recommendations are given for these fatty acids (see Chapter 10 for further description). Among other vitamin-like compounds should be mentioned myo-inositol and choline.

Most diets prepared for pigs are supplemented with vitamins. For macro-nutrients, such as energy and protein, it is possible to demonstrate a response to increments in intake, which can be evaluated against cost of increment. This is not possible for vitamins, because their cost is relatively low compared to the consequences of deficiency. Therefore, vitamins are usually supplied at levels higher than those shown to be required for optimal growth and absence of visual deficiency symptoms. The requirement for vitamins is defined as "the minimum amount per unit of body weight, or per unit of feed, that will prevent the occurrence of clinical signs of deficiency, abnormal physiological characteristics, and will satisfy the needs for optimum performance in terms of those characteristics which are of economic significance" [32]. Table 12.1 provides an overview of the provision of vitamins in pig nutrition according to Danish recommendations. This recommendation is assessed as the minimum requirement plus a safety margin considering factors that may influence the minimum requirement. These factors were bioavailability, content of growth inhibiting agents in the feed, interactions between single vitamins, and between vitamins and, for instance, trace elements. In the following section, we will refer to Table 12.1 for focus on differences and similarities between animal groups.

Table 12.1. Danish recommendations for vitamins, added amount per feed unit (modified after [36])						
	Unit	**Sows**		**Pigs**	**Pigs**	**Growers**
		Gestating	Lactating	3-5 wks, 6-9 kg	5-10 wks, 9-30 kg	30-100 kg
Fat-soluble						
Vitamin A	IU	8000	8000	8000	5000	4000
Vitamin D3	IU	800	800	800	500	400
Vitamin E	IU	40	165	140	140*	40
- as all-rac-alpha-tocopherol	Mg	36	150	130	130*	36
- as all-rac-alpha-tocopheryl-acetate	Mg	40	165	140	140*	40
- as RRR-alpha-tocopherol	Mg	27	111	94	94*	27
- as RRR-alpha-tocopheryl-acetate	Mg	29	121	103	103*	29
K3	Mg	2	2	2	2	2
Water-soluble						
Tiamine (B1)	Mg	2	2	2	2	2
Riboflavin (B2)	Mg	5	5	4	4	2
Pyridoxine (B6)	Mg	3	3	3	3	3
Niacin (B3)	Mg	20	20	20	20	20
Biotin (B7)	Mg	0.2	0.20	0.2	0.2	0.05
Pantothenic acid (B5)	Mg	15	15	10	10	10
Folic acid	Mg	1.5	1.5	0	0	0
Cyanocobalamin (B12)	mcg	20	20	20	20	20

* When using a diet from 20 to 30 kg, the amount of vitamin can be reduced to the same level as stated for growers. The 130 mg (as dl-alpha-tocopherol) per feed unit is documented from 6 to 20 kg.

3. Structure, sources, and biological function

Unlike carbohydrates, lipids and protein, vitamins are not merely building blocks or energy-yielding compounds, but are involved in, or are mediators of, biochemical pathways. For example, many types of vitamin B act as co-factors in enzyme systems, but it is not always clear how deficiencies are related to the failure of the metabolic pathway. It is beyond the scope of this chapter to go into details of vitamin biochemistry, and in order to go deeper into the understanding of the biological function of the vitamin, the reader is therefore encouraged to look into more detailed descriptions in books concerning nutrition and metabolism of vitamins. Hence, the present chapter will only provide a brief description of the chemical structure, the biological function of each vitamin and their deficiency symptoms as summarised in Table 12.2. The classification of vitamins into fat-soluble and water-soluble is not only valuable to indicate whether a vitamin is likely to be absorbed similarly to lipids or like other water soluble nutrients, but it also helps the chemist to decide on the best way to extract and analyse a particular vitamin in feed/food and biological tissues.

Table 12.2. Name, common natural sources and deficiency symptoms of vitamins in pigs.			
Vitamin	Chemical name (main action)	Sources	Deficiency symptoms
Fat-soluble			
A	Retinol (Sight, epithelial tissues)	Liver, egg yolk, milk fat	Eye disorders, impaired appetite, retarded growth
D3	Cholecalciferol (Calcium absorption)	Egg yolk, fish	Rickets (young/growing), osteomalacia. Enlarged joints, broken bones, stiffness of the jpints, occasionally paralysis. Reduced growth rate.
E	Tocopherol, tocotrienol (Antioxidant)	Green fodders, cereal grains, vegetable oils	Myopathy (muscle degeneration), cardiac disease (mulberry heart disease).
K	Phylloquinone, menadione (Prothrobin synthesis)	Green leafy material, egg yolk, liver, fishemeal	Not reported under normal conditions.
Water-soluble			
B1	Thiamine (Carbohydrate and fat metabolism)	Cereal grains, seeds, roots, leaves, fermentation products, animal products	Pigs are practically unable to suffer from thiamine deficiency. Loss of appetite, muscular weakness.
B2	Riboflavin (carbohydrate and amino acid metabolism)	Green plants, yeast, liver, milk (esp. whey). Cereal grain is a poor source.	Poor appetite witj consequent retarded growth, vomiting, skin eruptinos and eye adnormalities.
B5	Niacin (nicotinamin and nicotinic acid) (Hygdrogen transfer NAD and NAPD)	Liver, yeast, groundnut and sunflower meals	Poor growth, anorexia, enteritis, vomiting and dermatitis, esp. if diets are rich in maize.
B6	Pyridoxin (Amino acid metabolism)	Widely distributed in cereal grains, plants	Reduced feed intake, anaemia.
B3	Pantothenic acid (Acetate and fatty acid metabolism, Coenzyme A)		Slow growth, diarrhoea, loss of hair, scaliness of the skin and a characteristic goose-stepping gat; in severe cases, pigs are unable to stand.
B7	Biotin (Carbon dioxide transfer)	Oil seeds and vegetables, liver, milk, yeast	Foot lesions, alopecia (hair loss) and dry, scaly skin. Growing pigs: Growt rate and feed utilization are adversely affected. Breeding sows: Adverse influence on reproduction.
Folate	Folic acid (Metabolism of single carbon compounds)	Green leafy materials, cereals, extracted oilseed meals	Rare because of synthesis by intestinal bacteria.
Choline	(Component of lecithin)	Green leafy materials, cereals, egg yolk, yeast	Slow growth, fatty infiltration of the liver, swollen joints, uncoordinated movement, anorexia, splay legs.
Inositol	Myo-inositol	Plant foods	Fatty infiltration of the liver (other species)
B12	Cyanocobalamin (Propionate metabolism)	Microbial origin, liver	Piglets: Poor growth and lack of coordination of hind legs. Older pigs: Dermatitis, rough coat, suboptimal growth.
C	Ascorbic acid (Oxidation/reduction reactions)	Citrus fruits, green leafy vegetables	Scurvy, which is characterised by oedema, emaciation, diarrhoea. Failure in collagen formation. Resistance to infection.

3.1. Fat-soluble vitamins

3.1.1. Vitamin E

The term vitamin E refers to two groups of compounds, the tocopherols and the tocotrienols, and all forms of the vitamin contain two parts, a “head” (the chromanol part) and a “tail” (phytyl group) as shown in Figures 12.1A + B. The tail serves to anchor the vitamin in lipid membranes, in the lipids of adipose tissue and in the lipid surface and core of the lipoproteins. It is also important for binding affinity to the α-tocopherol transport protein (α-TTP). This tail contains 3 asymmetric carbons at positions 2’, 4’ and 8’, ie. 23 = 8 different structures (R or S) of each tocopherol are possible. Tocopherols produced in nature by plants all possess the RRR configuration, while synthetically manufactured α-tocopherol is an equal mixture of 8 stereoisomer forms of RRR-, RRS, RSR-, RSS, and SRR, SSR, SRS, SRR-α-tocopherol (Figure 12.1A).

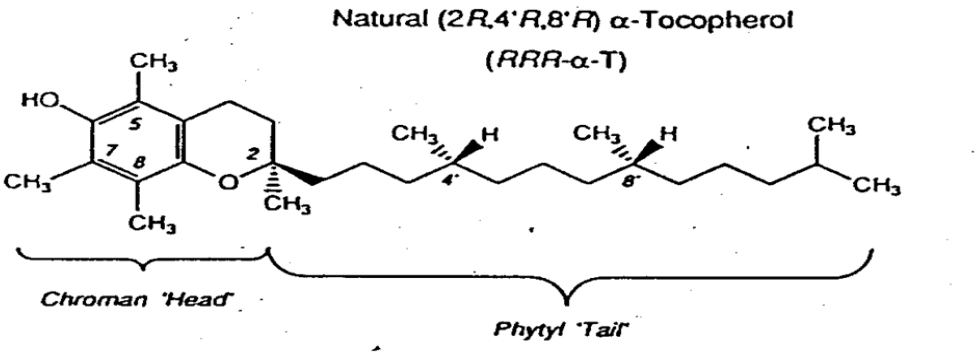


Figure 12.1A. α-tocopherol.

Eight naturally occurring forms of the vitamin are known, i.e., α, β, γ, and δ-tocopherol and tocotrienol, respectively (Figure 12.1B). Of these, α-tocopherol is the biologically most active form. The function of vitamin E is through its antioxidant[1] mechanism, as the chroman ring bears a hydroxyl group that can donate a hydrogen radical to other radicals. This means that vitamin E can bring a halt to a damaging chain reaction; i.e. the autoxidation of fatty acids. Grass and cereal grains are good sources of the vitamin, but the content in the different compounds varies. Wheat and barley contain mainly α-tocopherol and α-tocotrienol, but maize contains also an appreciable amount of γ-tocopherol.

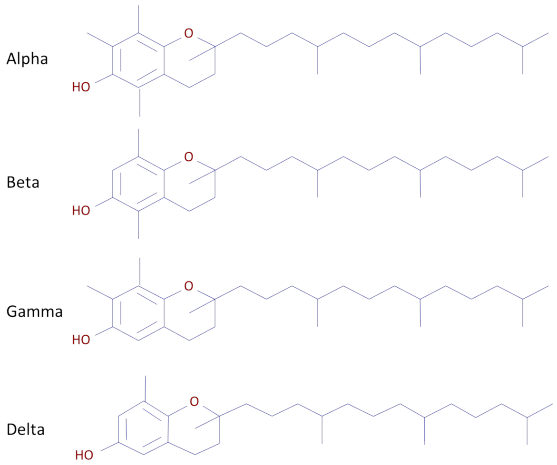


Figure 12.1B. Tocopherols.

Since the antioxidant activity of vitamin E is connected to the hydroxyl group, the stability during storage of the alcohol form is very low. Therefore, the major commercial form of vitamin E is the acetate bound form; all-rac-α-tocopheryl acetate. For absorption and biological function within the body, it is a prerequisite that the ester linkage is hydrolysed in the digestive tract, liberating the absorbable and biological active alcohol form of the vitamin. (Figure 12.2).

1 The term antioxidant is defined as any substance that, when present in low concentrations compared to those of oxidizable compounds, can delay or prevent oxidation of that compound [12].

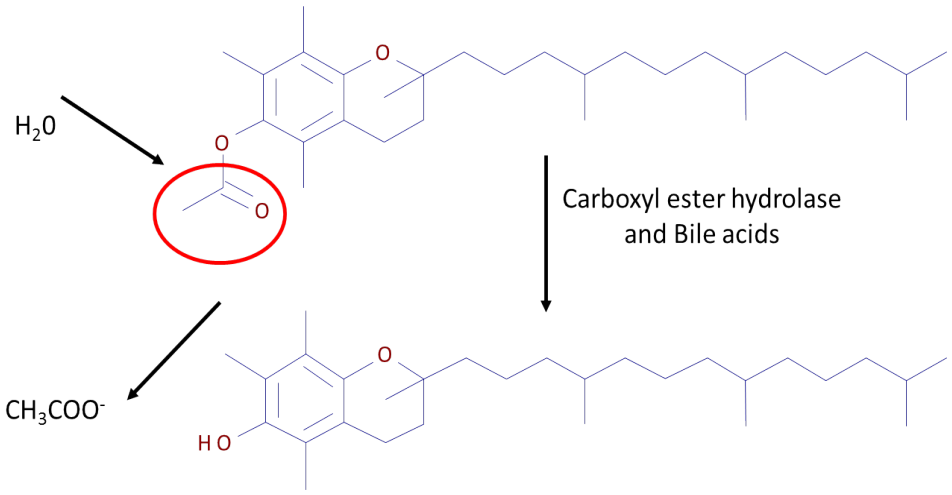


Figure 12.2. Hydrolysis of α-tocopheryl acetate to α-tocopherol.

The bioactivity of the stereoisomer form differs, and is much higher for the RRR-form as assessed by a fertility assay with rats. The bioavailability of the natural and synthetic form differs in pigs with a ratio of approximately 2:1. Thus the RRR stereoismer is normally predominant form followed by RRS, RSS and RSR, while the 4 2S forms (SRR, SSR, SRS, SRR) only contribute in small amounts in plasma, milk and tissues [19], [25] and this aspect has actually been taken into account during the revision of the Danish recommendations (Table 12.1). Symptoms of vitamin E deficiency in pigs are rare, and include impaired reproduction, Mulberrys heart disease, and muscle weakness (muscle dystrophy). It is likely that all biological problems and lesions that occur during deficiency arise from a failure to halt the rise in oxidised lipids, and it should at this point be stressed that whereas the antioxidative activity of the different stereoisomer forms of α-tocopherol is similar, only tocopherol, but not the acetate form, function as an antioxidant towards unsaturated lipids. The differences shown in Table 12.1 of forms of vitamin E reflect variations in bioavailability.

Vitamin E was also called “the fertility hormone” due to its essential function in reproduction. Reproductive defects in female animals involve a failure of the fetus to thrive. Most (older) pig studies have demonstrated an increased litter size at birth when vitamin E was supplemented in cereal grain-based diets. More recent research showed positive effect of increasing dietary levels of all-rac-α-tocopheryl acetate on sow reproductive performance, i.e., increased number of piglets born, and decreased incidence of mastitis, metritis and agalactia. In addition, the concentration of α-tocopherol in colostrum and milk increased with increasing dietary levels of vitamin E. Like other mammals, pigs have low placental transfer of α-tocopherol to the developing foetus, and are therefore born almost deficient in vitamin E making the provision of α-tocopherol through the sow colostrum and milk essential. Increasing the inclusion of dietary vitamin E to lactating sows are reflected in the pig plasma and tissue status at weaning.

One of the manifestations of a vitamin E deficiency in the male is testicular degeneration and inhibited sperm production. However, only few studies have investigated vitamin E requirements for male fertility in pigs. Semen is rich in unsaturated fatty acids, which increases the susceptibility to peroxidation and may cause structural damage to sperm and subsequently alter sperm motility. Dietary vitamin E may serve as an antioxidant in boar semen, whereas the addition of α-tocopherol to the ejaculate may not protect the sperm from peroxide damage because vitamin E needs to be incorporated in between the fatty acid esters of the phospholipids in the cellular membranes in order to exert efficient antioxidant protection, which is only possible through dietary means.

Research on vitamin E conducted by the authors has led to an increase in the recommendation of vitamin E for lactating sows and their progeny (VSP, 2005). This was the result of the critical vitamin E status observed at birth and at weaning of the progeny. The early post-weaning period is a critical state for vitamin E as a nutrient for growth and health. The shift from fat, rich sow milk containing the readily absorbable alcohol form of tocopherols that are easily absorbed together with the milk lipids to a low-fat weaning diet rich in dietary fibre and containing ester bound synthetic *all-rac*-α-tocopheryl acetate dramatically changes the digestive tract of the piglets. The enzyme responsible for hydrolysis of *all-rac*-α-tocopheryl acetate is pancreatic carboxyl ester hydrolase, an enzyme that is down-regulated post-weaning [17] due to the reduced lipid content in the feed. Although piglets nursing sows fed adequate or increased dietary vitamin E had higher reserves upon weaning, the tissue (heart and muscle) α-tocopherol declines rapidly during the post-weaning period [25]. Previous studies have indicated that the porcine liver has a very high short-term storage capacity for α-tocopherol [22]. Thus, as can be seen in Table 12.1, the recommended level of vitamin E for lactating sows and piglets (3-10 weeks) is much higher than for other groups. In a subsequent experiment with weaners fed increasing levels of different dietary all-rac-α-tocopheryl acetate (85, 150, and 300 mg all-rac-α-tocopheryl acetate/kg diet) at a 5% dietary fat level of varying fat sources, it was observed that the concentration of α-tocopherol in serum decreased during the first week after weaning, and that dietary vitamin E supplementation had no influence on plasma concentration before day 42 of age [24]. Thereafter, serum α-tocopherol concentration reflected the dietary vitamin E level and stayed between 1.5 and 2.0 mg/L when piglets were fed 150 and 300 mg all-rac-α-tocopheryl acetate/kg diet [24]. A plasma or serum concentration of 1.5-2.0 mg/L may be used as a guideline for establishing a satisfactory vitamin E status of the pig ([38]. Ideally, feeding emulsified natural α-tocopherol (RRR-α-tocopherol) to weaned piglets is the way to secure their optimal vitamin E supplement, but in practice the application of the alcohol form of vitamin E is a challenge due to the instable nature of the alcohol form.

3.1.2. Vitamin D

Vitamin D is closely associated with sunlight, and periodic exposure to summer sunlight can eliminate the dietary requirement for the vitamin. The pathway for vitamin synthesis requires a light-dependent step: the opening of an aromatic ring, resulting in conversion of the precursor of the vitamin to vitamin D.

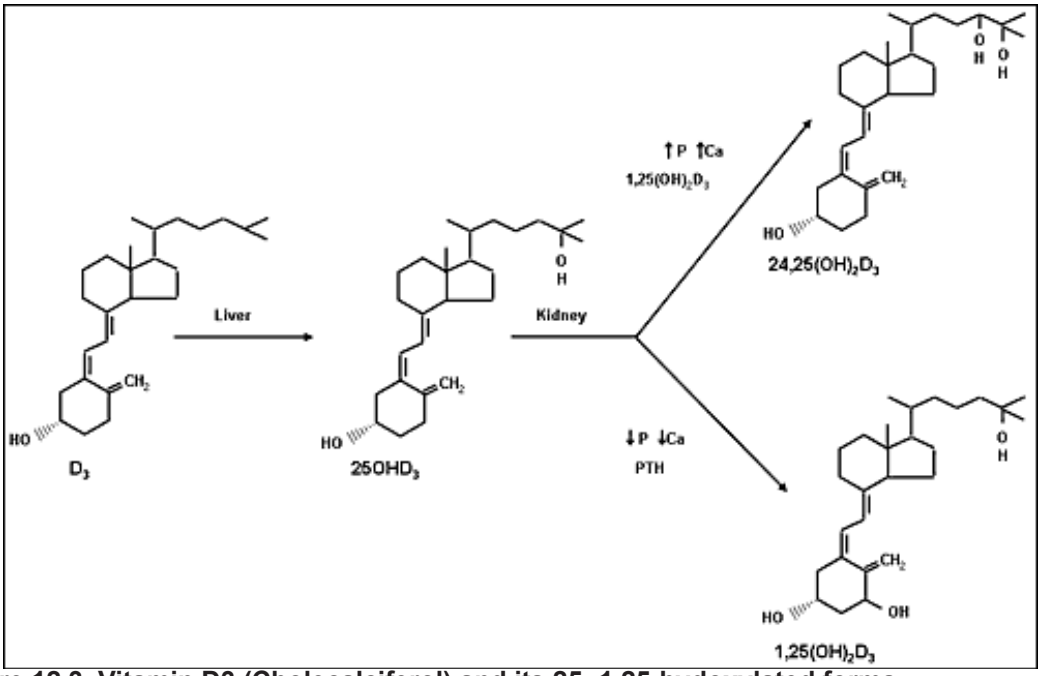


Figure 12.3. Vitamin D3 (Cholecalciferol) and its 25- 1,25-hydoxylated forms.

This is of course not an issue for pigs held in confinement indoors where exposure to sunshine is lacking. Most feed for pigs, with exception of fishmeal, contain little or no vitamin D, and the vitamin is generally supplied in the form of fish liver oils or synthetic preparations. Vitamin D exists as D2 (ergocalciferol) and D3 (cholecalciferol), but in terms of pig nutrition, vitamin D3 is the most potent one. Vitamin D3 is hydroxylated to 25-hydroxycholecalciferol (25-OH-D3) in the liver and this is the major circulating form in the body. The biological active form is further hydroxylated in the kidneys to 1,25-dihydroxycholecalciferol (see Figure 12.3). 25-Hydroxy vitamin D3 is present in plasma and used as a marker of vitamin D status at normal concentrations ranging from 15 to 60 ng/mL plasma. 25-Hydroxy vitamin D3 is commercially available in a synthetic form (HY-D®) and is immediately available to the blood stream upon intake and with a higher biological activity than vitamin D3. The hormonally active form of the vitamin, 1,25-(OH)2D3, is normally present at levels around a factor 100 lower than 25-OH-D3.

In humans, vitamin D deficiency is defined as 25-OH-D_3 levels below 25 ng/mL plasma. Prolonged deficiency can result in two diseases in pigs, rickets (piglets/growing), and osteomalacia (sows/boars), but this is very rare. Rickets is a disease of growing bone in which calcium and phosphorus are disturbed, and as a result the bones are weak and easily broken and legs may be bowed. Common symptoms include enlarged joints, broken bones, stiffness of the joints, and, occasionally, paralysis. In osteomalacia, the re-absorption of bone (calcium) already is laid down.

Vitamin D recommendation for sows during gestation and lactation is not based on scientific reports, and, in general, very little evidence is available regarding vitamin D in relation to reproduction in pigs. Thus, it is of major interest to improve the understanding of the role of vitamin D and its related metabolites in the breeding female pigs. Female fertility seems to be markedly reduced in vitamin D-deficient murine models. It has been postulated that local synthesis of 1,25$(OH)_2D_3$ may play a role in implantation and/or placentation either through the established immunomodulatory effects of 1,25$(OH)_2D_3$ or via the regulation of specific target genes associated with implantation. Overall the influence of vitamin D on the reproductive capacity seems to be linked to calcium-independent mechanisms. A recent study [23] was undertaken to obtain information on the dose-response pattern of two vitamin D sources, vitamin D_3, and 25$(OH)D_3$ with respect to early reproduction of reproducing female sows. As can be seen in Table 12.1, 800 IU vitamin D is the recommended level for gestating/lactating sows, whereas 200 IU is the minimum required amount according to NRC [32]. In experiment 1 [23], 160 gilts were randomly assigned from the first oestrus until d 28 of gestation to dietary treatments containing 4 concentrations of one of the two different vitamin D sources (200; 800; 1,400; and 2,000 IU•kg-1 from cholecalciferol, or corresponding to 5, 20, 35 and 50 µg•kg-1 feed from 25$(OH)D_3$ (Hy•D®), DSM Nutritional products A/S)). In a concurrent experiment, the same 8 dietary treatments were fed to 160 multiparous sows from the first day of mating until weaning. The reproductive performance of sows was not influenced by dietary vitamin D treatments, except for a lower number of stillborn piglets with the high doses of vitamin D (1400 and 2000 IU vitamin D giving 1.17 and 1.13 stillborn piglets per litter, respectively) compared with the low doses of vitamin D (200 and 800 IU vitamin D giving 1.98 and 1.99 stillborn piglets per litter, respectively). Furthermore, it was concluded that, irrespective of the dietary dose and form of vitamin D for the sows, very little vitamin D was transferred to the progeny. Thus, dietary treatment of lactating sows with high vitamin D doses was not an efficient nutritional strategy for increasing the piglets' vitamin D status [23]. Among domestic farm animals tested, baby pigs are actually born with the lowest plasma concentration of 25$(OH)D_3$, which increases their susceptibility to vitamin D deficiency. The fact that pigs differ from other husbandry may be partly due to the production systems as the concentration of plasma vitamin D and most of its metabolites in pigs exposed to sunlight was 2.2 to 20.3 times the concentration of pigs kept in confinement.

The fact that piglets are born with the lowest plasma concentration of 25-OHD_3 may predispose them to neonatal rickets, a condition of vitamin D deficiency associated with retarded skeletal growth and myopathy. Vitamin D deficiency reduces the retention of calcium, phosphorus and magnesium. As observed among reproducing pigs, the official recommendations for vitamin D for growing pigs seem to vary, i.e., from a suggested minimum requirement of 200 IU/kg diet for growing pigs (NRC) to the official British (BSAS, 2003) recommendations generally varying from 800

(for pigs until 60 kg liveweight) and 600 IU (from 60 to 90 kg liveweight)[2]. Danish recommendations vary from 800 (for piglets, approx. 6-9 kg), to 500 (for weaners, approx. 9-30 kg) and 400 IU (for pigs of 25-100 kg liveweight).

3.1.3. Vitamin A

Chemically, vitamin A is known as retinol (Figure 12.4), and in pig feed it may occur in two forms depending on their origin. In components of animal origin vitamin A occurs largely in the form of retinol or retinyl esters. A retinyl ester is a molecule of retinol, where the hydroxyl group is esterified with a molecule of fatty acid, normally acetate or palmitate. The other vitamin a source is carotenes (provitamin A) from plants. Plants do not contain vitamin A, but may be rich sources of provitamin A, i.e., carotenoids. More than 500 colourful carotenoids occur in nature, but only 50 of them can be used as precursors of vitamin A. Some carotenoids (e.g. lutein, lycopene, canthaxanthine) cannot be converted to vitamin A by mammals, and, being a "white fat" animal, pigs do not readily absorb carotenoids. The commonly used feed components in pig feed such as vegetable oils, lard, and cereals, are poor sources of vitamin A and provitamin A. However, alternative feed components of interest in order to stimulate immunity and health, such as algae, contain carotenoids, and fishmeal may contain appreciable amounts of vitamin A. Thus, in terms of vitamin A in pig nutrition, the commercial form to pay attention to is retinyl esters, which are released in the gut and rapidly hydrolysed to retinol. Retinol is stored in the liver in the form of retinyl esters. With mobilisation of the vitamin, retinyl esters are converted to retinol and released into the bloodstream as a complex with retinol binding protein. Vitamin A is very important for the health of many epithelial cells, including the eye. Thus, beside utilisation in the visual cycle, vitamin A also supports differentiation of epithelial cells and the viability of the reproductive system (foetal growth and vitality of testes). All three biological functions of vitamin A can be supported by dietary retinyl esters. Due to the liver storage of vitamin A, a proper assessment of the vitamin A status of a given animal would be by determination of the liver reserve where 30 ug/g liver is a typical value in pigs depending on the diets with which the pigs were produced.

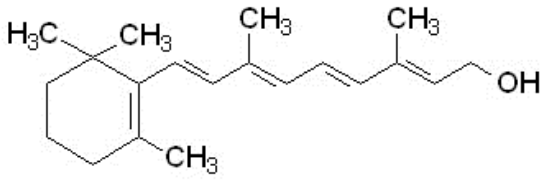


Figure 12.4. Vitamin A (Retinol).

The vitamin A reserves in the liver make it difficult to establish requirements especially in older animals (sows). As can be obtained from Table 12.1, the recommended level of vitamin A for growers is half of the level for reproducing pigs. Plasma retinol concentrations usually remain relatively constant over a wide range of dietary intakes and liver stores. If plasma retinol concentration begins to decline, dietary vitamin A absorbed by the liver is secreted directly into the circulation bound to retinol binding protein. Thus, determination of plasma vitamin A is a rather inaccurate method to use for determination of vitamin A requirements, and likewise with evaluation criteria such as weight gain and cerebrospinal fluid pressure. However, low plasma levels (< 0.20-0.25 µg/ml) indicate vitamin A deficiency. Hence, the requirements given by NRC [32] are rather broad ranking: during the first 8 weeks of life, 75-605 µg retinyl acetate/kg feed assessed by using various evaluation criteria. In growing-finishing pigs, the requirement varies from 35 to 130 µg retinyl acetate/kg when daily gain was used as criterion, and from 344 to 930 µg retinyl acetate/kg, when liver storage and cerebrospinal fluid pressure were used as the criterion [32]. However, vitamin A fortification in commercial starter diets is generally added in excess of NRC [32] standards as also reflected in Table 12.1.

2 Normer for Næringsstoffer (in Danish), 2002, 10. udgave, Landsudvalget for Svin, Kbhv., Denmark [21]

Vitamin A is essential for reproduction, although the role in this function is relatively unknown. Indeed mature sows fed diets without supplemental vitamin A may actually be able to complete gestation normally without experiencing vitamin A deficiency. Vitamin A is required for maturation of ovarian follicles; for the proper functioning of corpora lutea and epithelial cells of the oviducts, the uterine environment, and the cervix; and for embryonic development. It stimulates oestrogen synthesis in tertiary follicles and progesterone synthesis in corpora lutea. Beside a decline in general health, lack of retinol leads to a decrease in ovarian size and to testicular atophy. Research has focused on the effects of vitamin A injection(s) to sows to stimulate reproduction, and, more recently, the impact of dietary supplementation or injection of vitamin A and/or β-carotene before mating and during early gestation has been studied. Generally speaking, the results seem to be controversial when it comes to end points such as measurement of litter size at birth and weaning. However, when studying other parameters related to the biological function of the vitamin A in early gestation, vitamin A may affect the ovarian progesterone production, thus influencing the secretion of several proteins in the pig uterus that are very important for the process of conceptus. This mechanism is especially true in pigs because the porcine throphoblast does not invade the uterine epithelium; it rather remains in superficial attachment to the uterine surface. The presence of RBP that can transport vitamin A from the maternal uterine endometrium to the conceptus has been demonstrated in uterine secretions from pigs in the luteal phase of the oestrous cycle, and in the pig conceptus. A recent study [2] demonstrated that reduced vitamin A during conception and early gestation, but not in later gestation, was associated with increased within-litter uniformity in birth weight, and the mechanism was proposed to be ascribed to alteration in progesterone production. Moderate reductions in maternal vitamin A at either stage of gestation did not affect gestation rate, litter size, progesterone secretion or allometric relationships between foetal or neonatal organ and total body size [2].

Research on the possible specific role of β-carotene (and other carotenoids) has previously been hampered by the assumption that its sole function in animals is to provide vitamin A. Consequently, knowledge of the role of β-carotene (Figure 12.5) on reproduction as well as on immune responses is relatively scarce compared with the knowledge existing on vitamin A. Crossbred gilts injected weekly with 228 mg of β-carotene from mating to weaning had lower embryonic mortality, larger litter size, and heavier litter weight at birth and at weaning than did unsupplemented gilts [3]. This is in general agreement with the study of Coffey and Britt [9] who reported a linear increase in litter size at birth in multiparous sows injected once at weaning with increasing doses of β-carotene (0, 50, 100, or 200 mg of β-carotene). It was unclear whether this was due to increased ovulation rate or to decreased embryonic mortality [7]. Even though some studies demonstrate improved reproductive performance with supplemental β-carotene, it remains unclear whether β-carotene plays a direct role in regulating certain reproductive processes or whether it merely serves as a source of vitamin A [7]. Studies on the possible role of β-carotene on male reproduction are lacking.

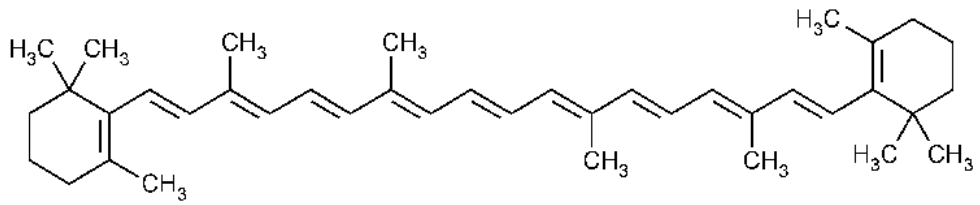


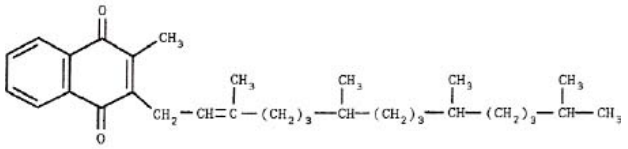
Figure 12.5. Beta-carotene (vitamin A precursor).

Vitamin A fortification in commercial starter diets is generally added in excess of NRC [32] standards. As stated earlier, the requirement depends on the assessment criteria. Therefore quite different requirements may be given according to the assessment criteria used. In weaners, no differences between three levels of vitamin A (2,200; 13,200, or 26,400 IU/kg) were obtained on performance with regard to a 35-day post-weaning period [8]. In addition, no effect on the perfor-

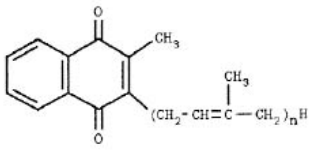
mance of growers was obtained with high doses of vitamin A (10,000-40,000 IU/vitamin A/kg feed) in comparison with the control treatment at 5,000 IU/kg feed [13]. However, it should be noted that high dietary levels of vitamin A could have detrimental effect on the young pig's vitamin E status during the post-weaning period, and could be detrimental to the antioxidant status [8].

3.1.4. Vitamin K

Vitamin K is essential for the synthesis of several proteins involved in blood clotting or coagulation. The vitamin K dependent proteins that are involved in regulating blood clotting are factor II, factor VII, factor IX, factor X, protein C, and protein S. Other vitamin K dependent proteins are uniquely involved in regulating bone growth (osteocalcin) and in regulating cell growth. All compounds exhibiting vitamin K activity possess menadione. Two forms of vitamin K exist naturally: phylloquinone and menaquinone. Both forms (Figure 12.6) are used with only slight modification in the body, ie. reduction to dihydrovitamin K. Phylloquinone (vitamin K_1) is synthesized in plants, whereas menaquinone-n (vitamin K_2) is synthesized by bacteria.



phylloquinone (vitamin K_1)



menaquinone-n (MK-n, vitamin K_2)

Figure 12.6. Viamin K.

Symptoms of vitamin K deficiency have not been reported in pigs under normal conditions, and it is generally considered that bacterial synthesis in the digestive tract supplies sufficient vitamin for the animal's needs, possibly during coprophagy (consumption of feces). A number of microorganisms are known to synthesize vitamin K. Drugs that affect the bacteria in the gut may depress the production of vitamin K. The current Danish recommendation of vitamin K is similar for reproducing animals, piglets and growers, and is based on the use of antibiotics, lack of coprophagy and the decreased stability of the vitamin. According to NRC [32] no data is available on which to base an estimate of the requirement of growing pigs (30-90 kg) or of breeding sows. Hence, no data is available to support varying requirement among animal groups, and no information is available on the requirement of vitamin K in diet to which antibiotics are not added.

3.2. Water-soluble vitamins

The vitamins included under this heading all dissolve in water and most of them (the vitamin B complex) are components of coenzymes. Unlike the fat-soluble vitamins, they are not stored in the tissues (except cyanocobalamin, B_{12}), and a regular supply from external sources is essential. As can be seen from Table 12.1, the recommended Danish levels of these vitamins do not differ between gestating and lactating sows, or between the different categories of the growing pigs.

3.2.1. Thiamine (vitamin B_1)

The cofactor form of thiamine (Figure 12.7) is thiamine pyrophosphate (TPP), which is also the main form of thiamine in animal tissues. TPP is released from dietary proteins during hydrolysis in the gastrointestinal tract and then hydrolysed to thiamine.

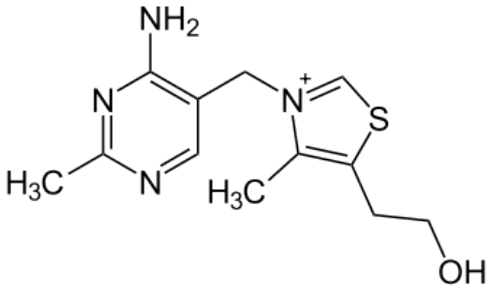


Figure 12.7. Thiamine (B1).

Most feedstuffs in pig nutrition contain natural thiamine, especially grain, and according to NRC (1998), diets based on grain and oily seeds may contain sufficient amounts of available thiamine to cover the requirement of pigs. Thus, supplementation of pig feed may not be necessary, but it should be mentioned that the thiamine requirement for pigs was assessed on rather old studies using pigs with less growth potential than today. In humans, thiamine deficiency is called beriberi, and in pigs, thiamine deficiency produces anorexia and weight loss, and pigs may vomit and experience respiratory problems.

Thiamine status can be assessed by direct tests involving the measurement of thiamine levels in the blood or urine, but can also be assayed by e.g. microbiological assays. The most reliable method for assessing thiamine status involves the measurement of red blood cell transketolase. This enzyme is measured with and without the addition of TPP to the enzyme assay mixtures. In dietary thiamine deficiency, synthesis of transketolase continues, but conversion of the apoenzyme to the holoenzyme in the cell is inhibited, resulting in the accumulation of the enzyme in the apoenzyme form. The measurement of erythrocyte transketolase activity and its percentage stimulation by thiamine pyrophosphate is a useful and sensitive method for assessing the thiamine status of pigs. It should be noted that the requirement for thiamine of pigs from weaning to slaughter is approximately four times higher according to this method than when based on the criterion of maximum growth rate only. Thus, given the higher growth potential of pigs today than when thiamine requirement was determined as referred to in NRC [32] using the criterion of maximum growth rate, the knowledge on the thiamine requirement for growing pigs may be rather uncertain. Likewise, the requirement for thiamine during reproduction is based on a very limited number of experiments, and there is no solid evidence from work with other animals that suggests the requirement for thiamine for reproduction to be substantially different from that for optimum growth. Only one experiment since NRC [32] has been reported regarding thiamine for pigs: Woodworth et al [39] demonstrated that dietary supplementation of thiamine up to 5.5 mg/kg (2.5 - 5 times the official recommendations [32] had no effect on growth performance of weaners as compared to unsupplemented diets.

3.2.2. Riboflavin (vitamin B2)

Free riboflavin rarely accumulates in the cell, and the vitamin in food and in the body occurs mainly in cofactor forms, i.e., flavin mononucleotide (FMN) and flavin adinin dinucleotide (FAD). Dietary FAD and FMN are hydrolysed by phosphatases or pyrophosphatases of the gut mucosa, which liberates free riboflavin. Riboflavin is essential in the metabolism of protein, carbohydrate and fat, and is therefore important for growth, including growth of the foetus. The FAD cofactors based on riboflavin are called flavins; enzymes using flavins as a cofactor are called flavoproteins. Natural riboflavin occurs in all animal raw products, and liver in particular is a good source. Although riboflavin (Figure 12.8) is present in grain, maize and soy products, the content in these

feedstuffs is low. Riboflavin is absorbed by the gut (main site ileum) and enters the bloodstream where close to half of it is loosely bound to serum albumin. The conversion of riboflavin to FMV is catalysed by flavokinase, and the conversion may occur during absorption through the gut mucosa or in other organs.

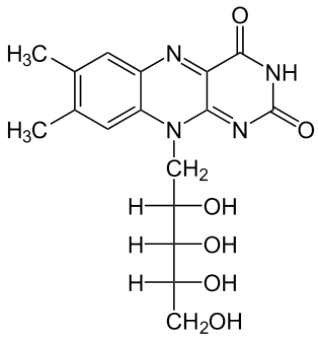


Figure 12.8. Riboflavin (B2).

Before 1988, research had not suspected a role for riboflavin in pig reproduction. However, it was shown that the sow's uterus secretes large quantities of riboflavin approximately one week after mating [30]. The physiological role of this is not yet known in detail. Divergent results have been obtained regarding the effect of dietary riboflavin during early gestation on litter size, and no definitive recommendations on riboflavin requirements in reproducing sows can be given. The most reliable way to assess riboflavin status is by a functional test, which involves the assay of glutathione reductase (EGRAC = erythrocyte glutathione reductase activity coefficient), using red blood cells as the source of the enzyme. This test, which is developed in human nutrition, has also been commonly used in pig nutrition along with traditional reproductive performance criteria. Although the EGRAC levels appear to be a reliable criterion for identifying riboflavin deficiency, there are some doubts as to its validity as a sensitive index of riboflavin efficiency. In fact, EGRAC values are not correlated with total riboflavin concentrations in the blood or liver of pigs, which means that the EGRAC values are probably not reliable in pigs. The natural content of riboflavin in feed is not sufficient to maintain the concentration of riboflavin in the blood in sows during early gestation and during lactation. In herds suffering from low farrowing percentage, riboflavin supplementation above Danish recommendations during early gestation may be beneficial. However, this remains to be documented.

3.2.3. Pantothenic acid (vitamin B5)

This vitamin (Figure 12.9) has two functions, partly in the biosynthesis of coenzyme A and partly in the synthesis of the cofactor of fatty acid synthase.

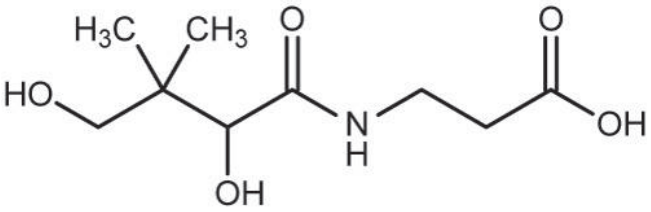


Figure 12.9. Pantothenic acid (B5).

Coenzyme A is used in a variety of reactions, for example Krebs cycle, fatty acid synthesis and oxidation, amino acid metabolism, ketone body metabolism, cholesterol synthesis, and conjugation of bile acids. Pantothenic acid is present in all plant and animal foods, and occurs mainly as coenzyme A. Coenzyme A does not readily cross cell membranes, including those of the gut. Dietary coenzyme A is hydrolysed in the gut lumen to yield pantothenic acid, which is readily absorbed. Levels of pantothenic acid in body fluids and feedstuffs can easily be measured by microbial assays, in which lactic acid bacteria are used as the test organism. In humans, a deficiency purely in pantothenic acid has probably never occurred except in controlled studies. Pantothenic acid deficiency is considered to be rare in practice because of the wide distribution of the vitamin. Studies with pigs have shown that consumption of a diet deficient in the vitamin results in a loss of appetite, slow growth, skin lesions, loss of hair, scaliness of skin, ulceration of the intestine, weakness and possibly death. In addition, a characteristic “goose-stepping” gait; in severe cases animals are unable to stand. Signs of pantothenic acid deficiency have also been produced in gilts and progeny provided a synthetic pantothenic acid deficient diet. The Danish recommendation for pantothenic acid is based on scientific information of NRC [31], and the given recommendation has not been changed [32]. No new information on pantothenic acid in relation to reproduction of sows has been reported since that time. However, with regard to piglets and growers, pantothenic acid has received some attention in recent years. In early weaned piglets, a linear response on performance was observed within the range of 0 to 120 mg/kg. The mechanism may be partition of energy from fat accretion towards protein deposition. However, other studies have not reported any response with dietary levels of pantothenic acid supplementation between 30 and 90 mg/kg.

3.2.4. Niacin (vitamin B3)

The term ‘niacin’ refers to both nicotinic acid and to nicotinamide. Niacin (Figure 12.10) in foods occurs mainly in the cofactor form, NAD (nicotinamide adenine dinucleotide), and the phosphorylated form, NADP (nicotinamide adenine dinucleotide phosphate), and their reduced versions.

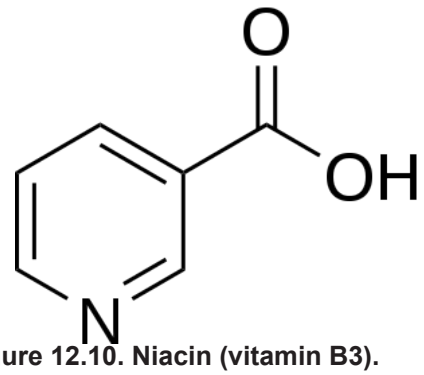


Figure 12.10. Niacin (vitamin B3).

Most feedstuffs for pigs generally contain a high content of niacin, but the bioavailability in grain such as barley and wheat is in general low, and almost all niacin in maize is bound in a form that is completely unavailable to the pig. However, niacin can be produced in tissues from tryptophan, and it is generally accepted that 1/60 of the tryptophan in the diet is converted to nicotinic acid and nicotinamide. This biosynthesis can take place in most animals, including pigs (except baby pigs under 3 days [32]. The requirement for the vitamin is therefore linked to the intake of this essential amino acid, and must be given in terms of “niacin equivalents”. Indeed, some plant-derived foods (and more so in animal-derived food), the tryptophan content is more important than the niacin content in providing “niacin equivalents”. For instance, the niacin content in wheat flour is 2.48 mg/1000 kcals, and the tryptophan content is 297 mg/1000 kcals, totalling 7.43 “niacin-equivalents”/1000 kcals [32]. Thus, estimation of niacin requirement is rather complicated because it is tightly linked to the tryptophan metabolism.

Niacin may also be synthesized in the gut of pigs: Dietary NAD is hydrolysed by the enzymes of the gut mucosa to yield nicotinamide. Dietary NAD can also be hydrolysed in the gut mucosa at the pyrophosphate bond to yield nicotinamide nucleotide, and both cofactor forms are broken down,

possibly by enzymes in the gut and in the liver, to yield nicotinic acid. Nicotinic acid or nicotinamide are used as feed supplements for pigs.

NAD tends to be an electron acceptor in catabolic reactions involving the degradation of carbohydrates, fatty acids, ketone bodies, amino acids, and alcohol. NAD is used in energy-producing reactions. The cytosolic NADP tends to be involved in biosynthetic reactions. In pigs, niacin deficiency may result in anorexia, reduced growth, diarrhoea, enteritis, vomiting and dermatitis. However, niacin deficiency appears rarely in pigs above 27 kg, and in general little impact of niacin supplementation has been obtained with regard to reproduction of sows. Lack of response in sows to niacin supplementation (33 mg/kg feed, i.e. 160% of the Danish recommendation) was explained by the natural content of niacin and/or tryptohan in the control diet [15]. Thus, a diet devoid of exogeneous niacin and containing a low concentration of tryptophan was sufficient to meet niacin requirements during gestation as well as lactation. According to this experiment, there is no rational argument for niacin supplementation to diets based on maize, soy or grain for sows. With regard to the impact of niacin supplementation to weaners and growers, more recent data is available, i.e., Real et al [33] concluded that the additional supply of niacin of up to 55 mg/kg improved gain:feed ratio and meat quality. In weaned pigs, a linear effect of dietary concentrations between 0 and 110 mg/kg was observed on gain:feed ratio during 5 to 7 weeks of age, while Matte et al [28] reported tendencies for increased feed intake from 4 to 6 weeks of age and an average daily gain from 6 to 10 weeks of age after supplementation of 15 versus 60 mg/kg of niacin. According to NRC [32] the minimum requirement for niacin to sows is 10 mg/kg, and 12.5-20 and 7-10 mg niacin/kg for pigs of 3-20 and 20-120 kg, respectively. In conclusion and based on the limited amount of data, no beneficial impact of niacin supplementation has been obtained with regard to reproduction or performance results of sows or slaughter pigs. However, with regard to piglets (3-20 kg) it seems more doubtful if the natural content of niacin would cover their requirement, and more recent studies (in comparison to [32]) seem to indicate that niacin supplementation above the minimum recommended level may exert some beneficial effects in terms of growth performance.

3.2.5 Pyridoxine (vitamin B6)

The parent form of this vitamin (Figure 12.11) is known as pyridoxine, the corresponding aldehyde derivative as pyridoxal, and the amine pyridoxamine, and the phosphorylated versions of these forms.

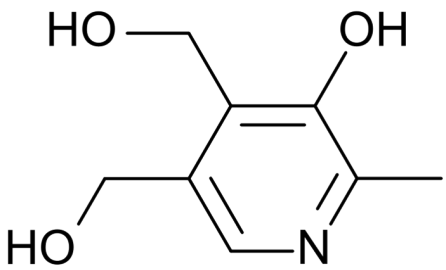


Figure 12.11. Pyridoxine (vitamin B6).

The most actively functioning one is the pyridoxal phosphate, which plays a central role as a coenzyme in the reactions by which cells transform nutrient amino acids into mixtures of amino acids and other nitrogenous compounds required for its own metabolism. These reactions involve the activities of transaminases and decarboxylases, and over 50 pyridoxal phosphate-dependent enzymes have been identified. The vitamin is believed to play a role in the absorption of amino acids from the intestine. Most of the body's vitamin B6 (70-80%) is present in muscle. Pyridoxine and its derivatives are widely distributed such as in cereal grains and milk. However, in piglets, pyridoxine concentrations in plasma are very low at weaning [28], probably due to the fact that sow milk is a poor source of vitamin B6 (approx. 0.40 ug/ml), and this amount would only represent roughly half of the daily requirement for growth in piglets. The symptoms of vitamin B6 deficiency

are not specific for this nutrient, because of the numerous enzymes requiring pyridoxal phosphate. Thus, a large variety of biochemical lesions are associated with B6 deficiency, and these lesions are concerned primarily with amino acid metabolism, and deficiency affects the animal's growth rate. In addition, pigs reduce their feed intake and may develop anaemia. In practice, vitamin B6 deficiency is unlikely to occur in farm animals because of the wide distribution of vitamins.

3.2.6. Biotin (vitamin B7)

Biotin (Figure 12.12), which is also called vitamin H, is a carrier of carboxyl groups in carboxylation reactions; i.e. a cofactor for four ATP-dependent carboxylases: acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, and beta-methylcrotonyl-CoA carboxylase. Biotin is produced by the gut microflora, which, in humans, has been estimated to supply half of our requirement. Formerly it was also anticipated that biotin synthetized microbiologically in the gut of pigs and that the natural content in feedstuffs was sufficient to cover a pig's requirement. Biotin is widely distributed in feed and food such as oilseeds, vegetables, liver, and milk. However, it is bound in different ways in the feed, thus the availability in barley and wheat is low, whereas biotin in maize and certain oilseed meals such as soy bean meal is completely available. Biotin can be precisely measured in the laboratory using microbiological assays, where the test organism is *Lactobacillus plantarum*. Biotin can also be measured by HPLC in body fluids, but breakdown products (such as bisnorbiotin) may interfere with the interpretation of results from HPLC, but not with microbiological assays. Young pigs in particular have a requirement for biotin. Clinical signs of experimentally-produced biotin deficiency have been described in piglets, growing pigs, and sows. These signs include dry skin with hair loss, the formation of scrabs and fissures, seborrhoeic eczema, transversal grooves on the tongue and white surface film, various foot lesions and characteristic movement and squatting postures.

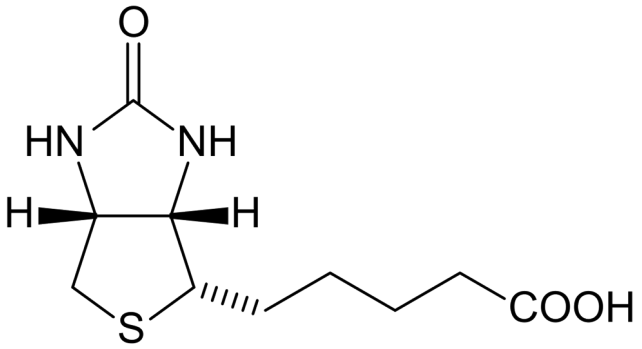


Figure 12.12. Biotin (vitamin B7).

Research has shown a good correlation between the concentration of biotin in the blood and the development of deficiency symptoms, and according to Jakobsen [16] the concentration of biotin in the blood of sows should be around 700 ng/l, which can be obtained by supplementation of 0.1 mg biotin/kg to a wheat/barley-based basic diet. Most results obtained regarding the supplementation of biotin above the requirement for pigs have addressed reproduction as the response, yet diverging results are seen. Lewis et al. [26] reported no effect of a dietary supplement of 330 ug/kg on litter size at birth, but an increase in the number of piglets weaned at 21 days of age. Only one study concerning biotin supplementation for reproducing sows has been reported since NRC [32]: Garcia-Castillo et al., [11], who found no effect on reproductive performance after massive biotin supplements of 10 and 28 ppm biotin versus 0.07 ppm. The requirement for biotin for growing pigs has never been assessed, and it has not been common practice to supplement the feed for growers with biotin except feed for piglets. Decreased fat quality, i.e., fat sources containing a high amount of peroxides may cause oxidation of biotin. In terms of the effect of biotin supplementation on foot lesions, the impact observed may depend on the length of the experimental period. The overall conclusion is that it is difficult to determine the biotin requirement for pigs and to estimate the optimal level.

3.2.7. Folate

Folate (Figure 12.13) is a water-soluble vitamin B. Folate is critically important for growth, and for this reason it is required in increased amounts in early gestation. The need for dietary folate remains elevated after gestation and during lactation because of the transfer of the mother's vitamin to the milk. Folate is a generic term referring to a family of related compounds. All of these compounds represent modifications of the simplest form of the vitamin, folic acid (pteroylglutamic acid, PteGlu). Folic acid is present naturally in most feedstuffs for pigs - particularly crushed soy bean has a high content. However, the content of natural folic acid in grain shows great variations, and the degree of utilisation in pigs is not known. Dietary folates consist mainly of folylpolyglutamates, and they are readily assimilated by the body and converted to the active cofactor form of the vitamin. The folylpolyglutamates of foods contain 4-7 residues of glutamic acid. The metabolic roles played by folic acid are closely linked to those of vitamin B12. Folates are used as cofactors in a series of reactions known as 1-carbon metabolism, which is involved in the synthesis of methionine, purine and pyrimidine bases. Therefore folic acid plays an important role in protein deposition and tissue synthesis. Folylpolyglutamates do not readily cross cell membranes. They are poorly transported by gut, liver, and other cells, and they also tend to be poorly transported by folate requiring microorganisms. Folate status can be assessed by measuring folate levels in serum or red blood cell; by histidine load test; and by haematological methods.

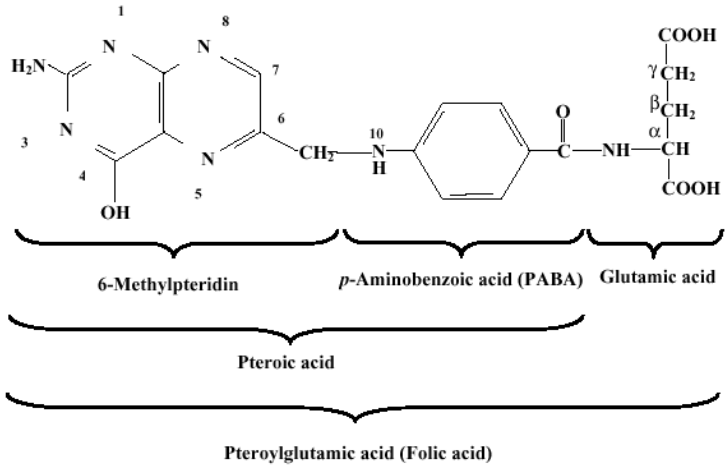


Figure 12.13. Folate.

According to NRC [32], there is only limited evidence of pigs' requirements for folic acid. For growing pigs, there is no dietary requirement as the needs are met through the feed and bacterial synthesis in the intestinal tract. Sows may have a need for dietary folic acid, at least under some conditions, although the evidence provides no basis for estimating this requirement. It is well-known that providing folic acid supplements to gestating sows increases prolificacy by roughly 10%. Supplements given during lactation were also efficient in increasing growth of piglets possibly through increased transfer of the vitamin through colostrum and milk. Folic acid acts directly on embryo development and indirectly by simulating the uterine secretion of growth promoters. The responses may depend on the parity of the sows. In conclusion, feed for sows is commonly supplemented with folic acid. On basis of recent literature on the beneficial impact of folate supplementation on reproduction, and on post-natal growth of pigs when provided to sows during lactation, NRC increased their recommendation for sows from 0.3 [31] to 1.6 mg folic acid per kg feed [32], which is in accordance with the current Danish recommendation of 1.5 mg/feed unit for reproducing pigs, whereas no supplementation is recommended for weaners and growers (LUS, 1998).

3.2.8. Vitamin B_{12} (cyanocobalamin)

Cobalamin is the term used to refer to compounds having C activity, as well as to related compounds. Vitamin B_{12} (Figure 12.14) is unique among all the vitamins in that it is the largest and most complex, and because it contains a metal ion, namely cobalt.

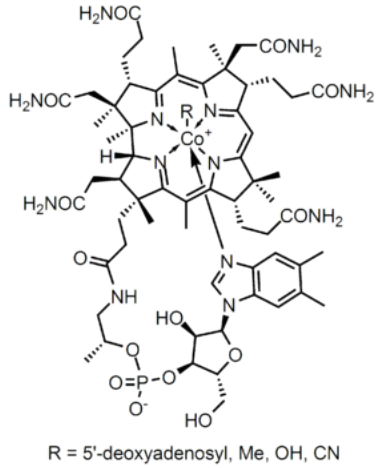


Figure 12.14. Cyanocobalamin (vitamin B12)

Along with vitamin D, vitamin B12 is the only vitamin generally absent from plant and vegetable foods. Before vitamin B_{12} can be absorbed from the intestine, it must be bound to a highly specific glycoprotein, termed the intrinsic factor, which is secreted by the gastric mucosa. The vitamin acts as a cofactor and functions in several important enzyme systems. These include isomerases, dehydrases and enzymes involved in the biosynthesis of methionine to homocystine. The main function of vitamin B_{12} is so interconnected with folic acid that it is difficult to distinguish a deficiency in one from that in the other without extensive testing. The major effect of B_{12} deficiency is impaired growth, particularly of rapidly growing cells such as immature red blood cells. Piglets grow poorly on vitamin B_{12} deficient diets and show lack of coordination of the hind legs. In older pigs, dermatitis, a rough coat and suboptimal growth are seen. B_{12} deficiency also results in the build-up of homocystine in the cell and bloodstream. As can be deduced from above, feed ingredients with plant origin are major sources of vitamins except for B_{12}, which, if present in plant materials, may only be so due to microbial contamination. The main natural sources are foods of animal origin, liver being a particularly rich source. Intestinal synthesis of the vitamin occurs in pigs.

3.2.9. Ascorbic acid

Ascorbic acid (Figure 12.15) plays an important part in various oxidation-reduction mechanisms in living cells. The vitamin is necessary for maintenance of normal collagen metabolism. As an antioxidant, ascorbic acid works in conjunction with α-tocopherol in protecting cells against oxidative damage caused by free radicals. Vitamin C can regenerate used vitamin E. The vitamin is required in the diet of only few vertebrates not including pigs. The synthesis of the vitamin is from glucose via glucoronic acid and gulonic acid lactone; the enzyme L-gulonolactone oxidase is required for the synthesis. Well-known sources of this vitamin are citrus fruits and green leafy vegetables, and as such pig feed may be deficient in vitamin C. Since farm animals are capable of synthesizing this vitamin, deficiency symptoms will normally not arise, and no recommendation of this vitamin is given (Table 12.1). However, it has been suggested that under certain stress conditions the demand for ascorbic acid increases to levels higher than that provided for normal tissue synthesis, and a dietary supplement may therefore be beneficial.

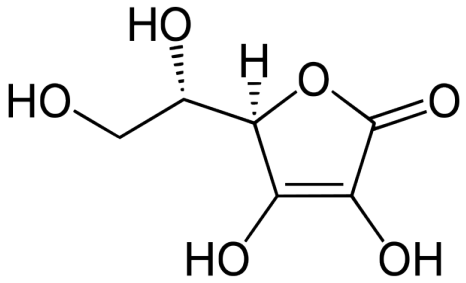


Figure 12.15. Ascorbic acid (vitamin C).

Sow milk contains 4-5 times as much vitamin C as cow milk, and sow colostrum has a higher content (68.4 and 57.9 mg/kg measured 0 and 68-76 hrs after parturition, respectively) than sow milk (45.3 mg/kg) [10]. This is a considerably higher amount compared with the concentration of fat-soluble vitamins. One may speculate that this is due to its capability of exerting antioxidant protection of fat-soluble vitamins in the milk fat.

3.2.10. Choline and inositol

Choline and inositol are not addressed in Table 12.1, but is mentioned in Table 12.2. Unlike the true vitamin B, choline is not a metabolic catalyst, but forms an essential structural component of body tissues. It is present in cereals and green leafy materials, and most choline is transported as part of lecithin phospholipid in chylomicrons and lipoproteins (see Chapter 10). In human tissues, most choline (as well as inositol) is part of phospholipid (and spingomyolin) present in intracellular and plasma membranes of all cells. It can be synthesized in the body tissues from methionine, and the dietary requirement for choline is, therefore, influenced by the amount of methionine in the diet. Thus, NRC [32] has based the dietary requirement of choline for pigs on the dietary level of methionine. However, choline is not listed in the Danish recommendations (Table 12.1) because of the *de novo* synthesis, and the consideration of not being a vitamin [16]. Likewise, myo-inositol, which is a particular stereoisomer of inositol, is not classified as a true vitamin B since it does not appear to play any part in enzymatic reactions; it can be synthesized in the body tissues and is present in animal tissues in amounts much greater than those usually associated with the true vitamin B. Deficiency in choline and inositol in other animal species results in slow growth and fatty acid infiltration of the liver, and dietary deficiency does not necessarily produce any deficiency symptoms in pigs. The induction of deficiency would appear to depend on other dietary factors such as total fat and composition of fatty acids ingested. Both choline and inositol have been referred to as “lipotrophic substances”, meaning that they are required for removal of fats from the liver.

3.3. Hypervitaminosis

Hypervitaminosis is the name given to pathological conditions resulting from an overdose of vitamins. Under ‘natural’ conditions, it is unlikely that farm animals will receive excessive doses of vitamins, although when synthetic vitamins are added to diets there is always a risk that abnormally large amounts may be ingested if errors are made during mixing. Experimental evidence shows that toxic symptoms can occur if animals are given excessive quantities of vitamins A or D. In pigs, toxic symptoms of vitamin A include rough coat, scaly skin, hyperirritability, haemorrhages over the limbs and abdomen, periodic tremors, and death. Symptoms corresponding with hypervitaminosis A (sudden lameness) appeared in pigs 3-4 days after introduction of feed with a vitamin A content ten times higher than the claim (195.000 IU/kg) [34].

Excessive intakes of vitamin D cause abnormally high levels of calcium and phosphorus in the blood, which result in the deposition of calcium salts in arteries and organs. Depending on the

exposure time, the upper dietary level of vitamin D_3 in pigs has been estimated at 33,000 and 2,200 IU/kg for less than and more than 60 days, respectively [29]. In terms of vitamin K, excessive doses may lead to depression in growth and anaemia. It should be noted that vitamin E toxicity has not yet been demonstrated in pigs; in fact dietary levels as high as 550 - 700 mg/kg have been fed to growing pigs without toxic effects.

It is noteworthy that overdosing only occurs with vitamins A and D, which from nature were never intended to be consumed orally in greater quantities. Therefore, the body has not developed feedback mechanisms to avoid excessive absorption. Thus, in a natural diet for pigs vitamin A will occur as pro-vitamin A (carotenes from green plants) and vitamin D will mainly be synthesized in the skin upon UV irradiation from the sun on the skin. Feedback mechanisms are well established for both these administration ways.

3.4. Requirements in relation to immunity and meat quality

The requirement for each vitamin was described in accordance with classical deficiency signs and non-specific parameters (for example impaired growth and feed intake, lowered reproduction rates) associated with deficiencies or excesses. Studies indicate that nutrient levels that are adequate for growth and meat production may not be adequate for normal immunity; for maximising the animal's resistance to disease or for optimal meat quality. We have attempted to depict this balance of optimum vitamin nutrition in Figure 12.16. Optimum vitamin nutrition can be defined as vitamin levels above minimum requirements to optimise genetic potential and improve immune status in the pigs, leading to an improvement in production and meat quality.

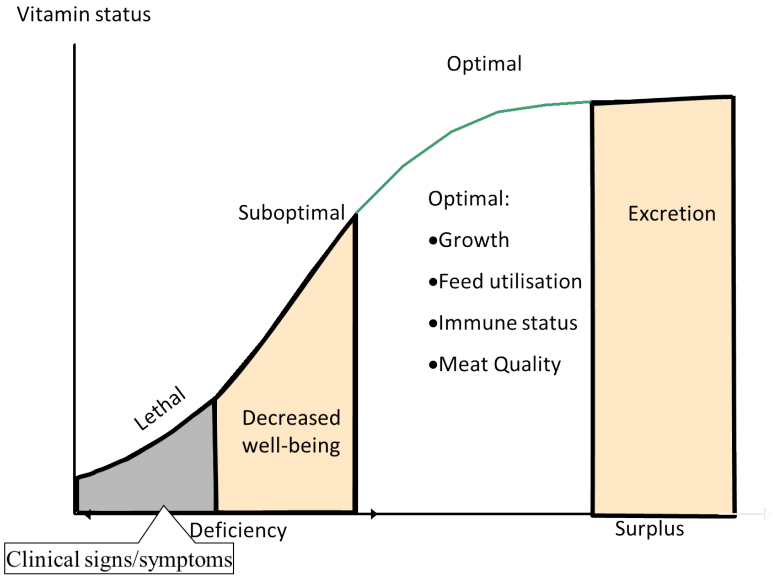


Figure 12.16.

Immune function: It is well-known from research in animals and humans that several of the vitamins are required for a normal immune function, and that vitamin deficiency would lead to impaired immune function. It is also well-known that mechanisms involved in modulation of immune responses can be undertaken by several of the listed vitamins. These mechanisms concern especially modulation of signal transduction in leukocytes (fat-soluble vitamins, and PUFAs (Chapter 10), and as substrates for the immune system (water-soluble vitamins). A third mechanism, protection against immunopathology, is exerted by the antioxidative vitamins. However, little information (and even less in pigs) is available on the vitamin levels required for optimal immune response. It is well researched in lab animals, chickens and humans, it is known that adequate amounts of vitamin E are necessary for normal immune function. The mechanism involved appears to be ascribed to its antioxidative activity and influence on stability of cell membranes, as well as prostaglandin synthesis and level of cortisol, and thereby its indirect effect on the lymphocyte function and inflammatory reactions. However, less information is available in pigs, and increased levels of vitamin E

are being investigated as potential immune modulators/enhancers. For pigs, levels above 3.0 mg α-tocopherol/L plasma may be required for maximum immune function.

Antibodies are proteins synthesized by the body for the purpose of recognizing foreign molecules and for inactivating the infectious organisms that are the source of the foreign molecules. The goal of recognizing this foreignness is to combat infecting viruses, bacteria, and protozoans. The immune system consists of several types of cells, of which the white blood cells called lymphocytes function to take up foreign proteins. Increased number of lymphocytes has been obtained in studies with growers given β-carotene by injection and in sows given β-carotene with the feed. In the sows, a higher level of antibodies was also registered. As mentioned, pigs have a poor absorption of intact β-carotene. However, β-carotene plays an important role in immunoregulation, and may therefore call for special attention with regard to weaners, whose immune system may be depressed. It has been shown that concentrations of plasma immunoglobulins (IgG) were higher in piglets born from gilts injected with β-carotene [3] although concentrations of immunoglobulins in colostrum had not changed. Increased mitogen proliferation has been reported in pigs supplemented with β-carotene [14]. Injection with β-carotene to pigs leads to an accumulation of the vitamer in all subcellular fractions of lymphocytes with lack of influence on concentrations of retinol or α-tocopherol in plasma [5], [6]. However, supplementation of β-carotene did not appear to influence the serum IgG concentration in sows and piglets [20]. Hence, the role of β-carotene supplementation on the immune cells and function of pigs remains to be elucidated.

Vitamin B also appears to be involved in the immune function of pigs. The ability of young pigs to produce antibodies in response to injected antigens was greatly reduced when fed a synthetic diet that was deficient in pantothenic acid, pyridoxine or riboflavin. Obviously, several vitamins seem to modulate the immune function of an animal, and deficiency may lead to impaired immune function, but whether requirements for optimal immune function differ from those of optimal growth is sparsely known.

Meat quality: Several studies have been performed with the overall purpose of improving pork quality through dietary vitamin E supplementation (for a review, see [18]). Lipid oxidation is one of the primary processes of quality deterioration in meat and meat products. The changes in quality are manifested in adverse changes in flavour, colour, texture, and nutritive value and by possible production of toxic products. Dietary supplementation of vitamin E above requirement levels has been found to be effective in reducing lipid oxidation in meat and meat products. The amount of vitamin E accumulated depends on muscle characteristics, and on level and duration of supplementation [18]. Actually, deposition of α-tocopherol does not reach saturation levels in muscle tissue of pigs fed 700 mg α-tocopheryl acetate. The major benefit attained is protection against oxidative changes, thereby improving storage stability. Other meat quality parameters affected are, for instance, meat colour and drip loss, though the efficiency of supplemental vitamin E to control colour deterioration in pork varies considerably. Relationship between endogenous vitamin E and drip loss reduction has been shown in pork, although the mechanism behind that needs to be fully explained. Thus, supranutritional (Supranutritional: 'supra'= above; i.e. the addition of vitamins/minerals in levels much higher than normally used in diets) vitamin E supplementation in finishing diets may be used as a strategic tool to optimise pork quality. However, dietary recommendations have not changed except for a general guideline on incorporation of 5 mg vitamin E/kg for each 1% dietary fat (above 3% dietary fat).

Recently, research on vitamin D for growing pigs has focused on the relationship with meat quality, as it could be expected that high amounts of vitamin D_3 to finishing pigs would increase muscle calcium, and subsequently improvement of meat tenderness was investigated. Wiegand et al. [37] fed supranutritional levels of vitamin D_3 (250,000 or 500,000 IU/per day) to finishing pigs three days prior to slaughter, and although the high dietary supplementation resulted in elevated plasma calcium concentrations, no improvement was observed on pork tenderness. On the other hand, supranutritional levels of supplemental vitamin D_3 improved pork colour and increased pH, but muscle calcium concentrations were not affected. However, one should realise that high doses of

vitamin D are dangerous. Although seldom reported in pig production, high doses of vitamin D may result in permanent deposit of minerals in heart, lungs and kidneys, as well as other symptoms of toxicity reported for humans. This may not be relevant when using cholecalciferol, but hazards of high intake of its active metabolite cannot be excluded.

As can be obtained from common food tables [35], pork and pork products are contributing sources of vitamin A, D, C, thiamine, riboflavin, B6, and folate. Some loss of these vitamins may be expected during the heating of the meat, e.g. 20% for vitamins A, C, B_6, and folate, but 60% for thiamine is lost during boiling and grilling, whereas vitamin D is in general heat-stable. One could therefore take into consideration the improvement of the nutritional quality of pork by feeding supranutritional vitamin levels. For vitamins such as vitamin D, which is not excreted, but stored in the tissues, this would actually be a possible nutritional strategy. On the other hand, the injection of retinol to optimise reproduction performance of sows actually increased the content of vitamin A in the liver to such an extent that pregnant Danish women were recommended not to eat products containing pig liver. For comparison, the content of vitamin A in most pork is 0 or very limited, whereas the content in raw pig liver can be as high as 14125 retinol equivalents/100 g, and in liver pate ("leverpostej") 3850 retinol equivalents/100 g. The recommendation of the daily vitamin A intake for a pregnant woman is 800 retinol equivalents.

4. External factors affecting vitamin requirements and vitamin utilisation

As a starting point, it can be stated that regardless of optimal performance and physiological status, pigs require that feed formulation contain all essential vitamins as listed in Table 12.1. However, there are certain management and production factors that may either increase or reduce the need for vitamin provision. The Danish recommendations for vitamins for pigs describe the amount of each single vitamin that should be added to a diet without taking into consideration the natural content of vitamins in feed ingredients. In some production systems, such as organic production systems, natural vitamin sources and factors influencing the stability and bioavailability may be of interest. In addition, many interrelationships of vitamins with other nutrients exist that consequently affect requirements. For example, prominent interrelationships exist for vitamin E with selenium, vitamin D with calcium and phosphorus, choline with methionine, and for niacin with tryptophan. Besides, vitamin antagonists (antimetabolites) interfere with the activity of various vitamins. In the following, we will consider external factors of importance when considering vitamin nutrition of pigs.

4.1. Storage and feed processing

As mentioned above, many typical feed ingredients for pigs contain vitamins, but the levels will vary because of crop location, fertilisation, plant genetics, plant diseases and weather. Harvesting conditions often play a major role in the vitamin content of many feedstuffs. Organic cultivation systems may increase the vitamin content of some feedstuffs although this remains a controversial question. Processing and storage of premixes and feeds may cause loss of activity because of stress factors such as humidity, pressure (pelleting), friction (abrasion), heat, light, oxidation-reduction, rancidity, trace minerals, pH and interactions with other vitamins, carriers, enzymes, and feed additives. Humidity is the primary factor that can decrease the stability in premixes and feedstuffs, and is even more stressful than temperature. During the storage of moist grain in silos, vitamin E activity can decline markedly; a reduction in vitamin concentration from 9 to 1 mg/kg DM has been reported in moist barley stored for 12 weeks. In terms of vitamin A, the long unsaturated hydrocarbon chains in carotenes (and vitamin A) are easily oxidised to by–products that have no vitamin potency. The oxidation process is enhanced by heat, light, moisture, and the presence of heavy metals. Consequently, foods exposed to air and sunlight rapidly lose their vitamin A potency, and so large losses can occur during the sun-drying of crops. Fresh grass is an excellent source of β-carotene, but the concentration is halved during ensilage. Likewise for vitamin E: the rate of

oxidation of natural tocopherol is higher in high-moisture corn than in low-moisture corn due to increased peroxidation of the lipid. Vitamin K is relatively stable under normal temperatures, but is rapidly destroyed when exposed to sunlight. Riboflavin is readily destroyed by either visible of ultraviolet light, and vitamin C and folic acid can also be destroyed by light and ultraviolet light, respectively.

Pelleting of feeds involves four elements; heat, friction, pressure and humidity that are applied in combined action and are destructive for a number of vitamins. Friction may erode the coating that protects several vitamins and reduces vitamin crystals to smaller particle size. The use of increased pelleting temperatures by feed manufactures in order to avoid Salmonella and increase digestibility may lead to increased vitamin degradation as increasing pelleting temperature generally enhances redox reactions and destroys vitamins. Thus, increasing pelleting temperatures from 77 °C to 88 °C may decrease the stability of many commercial vitamin products from 90-100 % to 90-95 %, whereas pelleting at 93 °C may further decrease the stability to 85-90 % (vitamins A, D, E, thiamine, niacin, and biotine), but even less for vitamins K,C, B12, folic acid and pyridoxine. In addition, processing of certain feed ingredients, and reduced quality of feed or feed ingredients can exert a negative influence in terms of vitamin nutrition. The use of rancid fat in feed inactivates biotin and destroys vitamins A, D, and E and possibly others. The presence of mycotoxins in feed increases the requirements for fat-soluble vitamins and other vitamins (for example biotin, folic acid and possibly others).

4.2. Management and stress factors

Today's intensive farm management, which is characterised by high levels of productivity, calls for intensive anabolism for optimal life processes such as muscle growth, gestation and lactation. Selection for faster growth may allow animals to reach much higher weights at much younger ages with less feed consumed. Intensified production increases stress and subclinical disease level conditions because of higher densities of animals in confined areas. Stress and disease conditions may increase the basic requirement for certain vitamins for pigs as for other animals. Information is lacking regarding the effect of management practice and husbandry methods, or breed of pigs, on the requirement for vitamins.

Vitamin provision for gestating and lactating sows and its efficient transfer to foetuses and piglets are particularly critical issues, because pigs are entirely dependent on the transfer of vitamins from their dams. Prolificacy due to intensive genetic selection programmes has led to a considerable increase in ovulation rate, but at the expense of embryo survival at the beginning of gestation. Several fat-soluble and water-soluble vitamins have been identified and related to physiological events in the first semester of gestation, in which embryo losses may represent 15-40% of the initial percentage of fertilized ova, and optimal embryo development relies on the so-called foetus-maternal dialogue. When considering a 'pig-life', it should be mentioned that the combined gestation and lactation periods (approx. 135-140 days) are as long as the rest of the post-weaning life of a slaughter pig (130 days), thus emphasising the importance of an adequate maternal transfer of vitamins. However, the fat-soluble vitamins, A, E, and D, are poorly transferred in the utero, whereas vitamins C and B_{12} are transferred.

The post-weaning period may be critical especially in terms of fat-soluble vitamins because of limited fat-absorption capability, i.e. the apparent digestibility of fat by suckling piglets is high, but at weaning it decreases to 65-80%. Fat-soluble vitamins may fail to be absorbed if digestion of fat is impaired. In addition, the requirement for vitamin E increases with a high dietary level of polyunsaturated fat. Furthermore, weaning age has declined over time to increase sow productivity. In sow milk, vitamins A and E are present in forms that are easily absorbed in the small intestine, whereas weaned pigs are served feed containing the commercial vitamin forms being acetate bound for vitamins E and A. These forms must be hydrolysed before absorption, which may be limited in pigs (see also Fig. 10.2 in Chapter 10). Besides, the two vitamins may compete with each other with regard to the limited amount of hydrolytic enzymes. In general, there is a greater relative growth re-

sponse for pigs, particularly for muscle tissue, during the post-weaning phase, which thus requires more vitamins. In addition, diseases or parasites/pathogens affecting the gastro-intestinal tract will reduce intestinal absorption of vitamins, both from dietary sources and those synthesised by the microorganisms. If the bugs cause diarrhoea or vomiting, this will also decrease intestinal absorption and increase requirements. Stress factors may increase the utilisation of several vitamins considerably, meaning that depots of e.g. vitamin E may be emptied within a day instead of 5 days. In addition, the requirement for ascorbic acid during post-weaning stress becomes greater, which can be provided for by normal tissue synthesis, and a dietary supplement may be beneficial. However, another strategic tool for vitamin supplementation to pigs during the post-weaning period would be to increase the vitamin content of the sow milk through supplementation of the lactation feed.

In contrast to the gestation, lactation and weaning periods, the finishing period does not seem to be critical in terms of vitamin nutrition. It has been proposed to withdraw vitamin supplementation during the finishing period in order to reduce feed costs. In this case, the animal relies on the vitamins in the tissue reserves. The fat-soluble vitamins can be stored in reserves when the dietary provision is in excess of the animals' requirements. However, vitamins B (except for B_{12}) and C are water-soluble and are not stored in substantial amounts. Although growth performance may not be altered in case of vitamin withdrawal in finishing feed, tissue reserves are depleted enough to affect concentrations of vitamins in meat and consequently the nutritional value of pork, and the quality of the pork especially during processing.

4.3. Production systems

It is obvious that the type of production system may influence åigs' requirement for vitamin supplementation of the feed. For the vitamin B complex, it is known that under the current livestock housing conditions, including the widespread use of slatted floors, the coprophagia behaviour is negligible and cannot provide a reliable supply of vitamin B. However, in outdoor systems and/or free-ranging systems, the contribution of several types of vitamin B synthesized by the microflora and excreted in the faeces may actually reduce the need for supplementation of vitamin B to the feed. In addition, the increasing use of roughage for gestating sows order to diminish aggression between sows may increase the microbial activity and thereby the synthesis of vitamin B. The allowance of sunlight may furthermore increase the vitamin D status of pigs kept in outdoor facilities during summer time (May – September). However, yet it has not been investigated if the de novo synthesis of vitamin D increases in pigs exposed to sunlight.

With regard to organic pig production, supplementation of substances such as vitamins should have a natural origin, and ideally 100% of the diets should be organic. In organic production, synthetic vitamin forms are allowed for monogastric animals only if identical to natural vitamins, and no GMO-produced vitamins may serve as additives in organically raised animals. However, there are differences depending on the standards (EC, 2007, 2008; IFOAM, 2005, USDA NOP 2008). The EC requires the vitamins to be identical to the natural vitamins and the IFOAM (2005) only allows synthetic vitamins when natural sources are not available in sufficient quantity and quality. The USDA NOP 2008 permits the use of synthetic vitamins when approved by FDA. Thus, a particular problem is the supplementation with vitamin E, which is produced chemically as a racemic mixture of eight stereoisomers of which only one has the natural configuration, and with vitamin B_{12}, which is mainly produced via bacterial fermentation processes conducted with genetically modified microbial strains.

5. Conclusion

There is no doubt that vitamins are essential for growth, maintenance and health of the pigs. Although the risks of a vitamin deficiency are now practically non-existent, the determination of the optimal levels for productivity of pig production remains a challenge. Besides the classical economic criteria – reproductive and growth performance – health, immunity and meat quality are critical issues as well the optimisation of the vitamin intake. The requirement for vitamins is critical during certain periods of a pig's life, especially during early reproduction, at birth and at weaning. However, little scientific information is available regarding the actual vitamin requirements, and the official vitamin recommendation is based on the use of antibiotic growth promoters and on housing in conventional production systems. In outdoor systems and/or free-ranging systems, the requirement for vitamins B and D may be reduced, and it may for most vitamins be possible to obtain 100 % natural vitamin nutrition to comply with organic production standards. However, information on the appropriate feed sources and the maintenance of stability of the vitamins remains critical focus points for further investigation. Although it has been 100 years since the the term "vitamin" was defined, there is still a lot to be learned and explored before the optimal vitamin nutrition for pigs can be assessed.

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