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VITAMINS IN RABBIT NUTRITION : LITERATURE REVIEW AND RECOMMENDATIONS

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ABSTRACT : Vitamins are classified in a total of 13 substances or groups of substances. Four vitamins are fat-soluble (vitamin A, D E, and K) and the nine others (vitamins of the B-complex and vitamin C) are water-soluble. The water-soluble vitamins and vitamin K are normally synthesised by the rabbit's digestive flora; but in cases of high risk of digestive disorders (*e.g.* just after weaning), dietary supplementation may be advisable. In addition, a vitamin C supplementation (25 to 30 mg per rabbit and per day) can help the animal in stress situations (heat stress, ...). The vitamin A requirement is largely satisfied if the diet contains 10 000 IU vitamin A per kg or 30 ppm of β-carotene. Additional provision of vitamin A is unnecessary for growing rabbits and may be toxic to the foetus in pregnant does. The dietary recommended vitamin D level is 800 to 1000 IU/kg. If the dietary concentration is greater than 2000 IU/kg, abnormal calcification of soft tissues (aorta, kidneys, ...) is generally observed. The recommendation for vitamin E is 50 mg/kg. A diet that contains only 15 mg/kg of vitamin E induces deficiency symptoms (muscular dystrophy, sudden death, reproduction disorders, ...). A massive introduction of vitamin E is not toxic but may improve rabbit meat shelf-life, by reduction of the rate of lipid oxidation

RÉSUMÉ : Les vitamines dans la nutrition du lapin : Revue de la littérature et recommandations.
Les vitamines sont classées en 13 substances ou groupes de substances. Quatre vitamines sont liposolubles (A, D, E et K) et les 9 autres (groupe B et vitamine C) sont hydrosolubles. Les vitamines hydrosolubles et la vitamine K sont normalement synthétisées par la flore digestive. Cependant en cas de risque de trouble digestif, comme après le sevrage, une supplémentation des aliments peu être conseillée. En outre un apport de vitamine C (25 à 30 mg par lapin et par jour) peu aider les lapins en période de stress (chaleur, ...). Les besoins en vitamine A sont largement couverts par un aliment contenant 10 000 UI de vitamine A/kg ou 30 mg de β-carotène. Une distribution supplémentaire de vitamine A ne présente aucun intérêt pour les lapins en croissance et peut être toxique pour les fœtus des lapines gestantes. Les besoins en vitamine D sont correctement couverts par une ration en contenant 800 à 1000 UI par kg. Si l'aliment contient plus de 2000 UI/kg, une calcification anormale des tissus mous (aorte, reins, ...) peut être observée. Les besoins en vitamine E sont correctement couverts par un aliment en contenant 50 mg/kg, mais un aliment contenant seulement 15 mg de vitamine E par kg est carencé (dystrophie musculaire, mortalité brutale, troubles de la reproduction, ...). A l'inverse, un apport massif n'est pas toxique mais peut améliorer l'aptitude de la viande de lapin à la conservation, par réduction de la vitesse d'oxydation des lipides.

Vitamins are organic substances without energetic value, but necessary for the metabolism of animals or human organisms. These substances are not synthesised by the organism itself in adequate quantities. For this reason, vitamins must be provided by feeds or through the intestinal flora activity. They act at very small concentrations mainly as co-enzymes or co-enzyme precursors, but are never incorporated as constitutive part of the body There are no chemical similarities between vitamins and the classification is based on their biological activity and only one chemical parameter : solubility in water or in lipids.

Vitamins are classified in a total of 13 substances or groups of substances. The main molecules with vitamin activity are listed in table 1. Vitamins are named with letters and an additional number when necessary, according to the order of their discovery. The missing numbers correspond to activities which were misinterpreted (different types of activity for the same molecules) or molecules which are normally synthesised by the organism.

The most common classification system is based on solubility : 9 vitamins are water-soluble, the B-complex vitamins (or B-group) and vitamin C, and 4 vitamins are fat-soluble (i.e. vitamins A, D, E and K). It is of great importance to consider this property for the conception of dietary vitamin supplies as for the interpretation of site and process of intestinal absorption.

Only vitamins A, E, B_{12} and folic acid are stored in the body . Vitamin A is stored in liver, vitamin D and E

Table 1 : Main molecules with vitamin activity (in bold character the main common names)

Vitamin Name	Chemical name of molecules
Vitamin A	Retinol – retinal - retinoïc acid - (beta-carotene = pro vitamin A)
Vitamin D	Ergocalciferol (D_2) - cholecalciferol (**D_3**)
Vitamin E	Alpha-tocopherol - beta-tocopherol - gamma-tocopherol - ....
Vitamin K	Phyloquinon (K_1) - menaquinone (K_2) - menadione (K_3)
Vitamin B_1	Thiamin
Vitamin B_2	Riboflavin
Vitamin B_3 or PP	Nicotinic acid - **nicotinamid - niacin**
Vitamin B_5	**Pantotenic acid**
Vitamin B_6	Pyridoxine
Vitamin B_8 or H	**Biotin**
Vitamin B_9	**Folic acid** - pteroylglutamic acic - pteroïc acid familly
Vitamin B_{12}	Cyanocobalamin - aquocobalamin - hydroxocobalamin
Vitamin C	Ascorbic acid - dehydroascorbic acid

in fat tissues and in muscles, but vitamin E is also stored in liver, as vitamin B_{12} and folic acid.

WATER SOLUBLE VITAMINS

In the rabbit, the digestive flora synthesises great amounts of water-soluble vitamins, mainly in the caecum. One part of these vitamins is available for the rabbit organism through direct absorption in the distal parts of the intestine, but the greatest quantities of vitamins are incorporated into the bacteria. These vitamins are absorbed in the rabbit small intestine after destruction of the bacteria ingested with soft faeces. It's one of the main benefits of caecotrophy. By this way, rabbits receive all B-complex vitamins and vitamin C necessary for maintenance and normal production.

B-complex vitamins

Experimental works published in the second part of the 20th century (see review by LEBAS in 1969) have demonstrated that fast growing rabbits may have a positive response to some vitamin B dietary additions, such as vitamin B_1 and B_6 (1-2 ppm), vitamin B_2 (6 ppm) and niacin addition (30-60 ppm). On the contrary, it has been impossible to provoke deficiencies in folic or pantothenic acid, as in vitamin B_{12} (HUNT and HARRINGTON, 1974). Nevertheless, these results were obtained with diets that can be considered as unbalanced today. For this reason, some new experiments are necessary to study vitamin B dietary needs of new rabbit lines with high productivity.

For choline, a dietary minimum was proposed by HOVE *et al.* (1957) : 0.12% of the diet. But their results were established with semi-synthetic diets that are known now to be unbalanced. If the ration provides sufficient amounts of methionine and folic acid, the choline needs of the organism are covered by transmethylation of methionine, and then choline addition in the feeds is not necessary. Nevertheless, as a precaution, MATEOS and DE BLAS (1998) suggested including 200 ppm of choline in commercial rabbit diets.

If a rabbit suffers from digestive disorders, production and ingestion of soft faeces are stopped. In this case, vitamins synthesised by the digestive flora are less available to the rabbit. Vitamin B storage in the organism is significant only for vitamin B_{12}. For this reason, rabbits with digestive disorders are more susceptible to water soluble vitamin deficiency, than healthy ones, and that's why it might be beneficial to add B-complex vitamins in the feeds of rabbits at risk of digestive disorders such as weanlings (LEBAS *et al.*, 1998). However, MATEOS and DE BLAS (1998) in their chapter on vitamin recommendations, emphasized that such an addition is not based on experimental results but only on deductive reasoning and practical observations in commercial rabbitries.

Table 2 : Vitamin C final content of some organs of rabbits receiving various sources of vitamin C, from 6 to 31 weeks of age : 0 or 50 mg/day (HARRIS *et al.*, 1956)

Ascorbic acid mg/100 g fresh tissue	Source of dietary vitamin C		
	None (control)	Ascorbic acid	Cabbage
▪ Adrenals	236	246	363
▪ Liver	16	19	24
▪ Blood	0,9	1,4	1,5

Vitamin C

A feed without any detectable vitamin C allows normal development of rabbits studied between 6 and 31 weeks of age (HARRIS *et al.*, 1956). In this experiment, vitamin C content of organs increased with age whatever the vitamin C supplementation (none or 50 mg per rabbit and per day in the form of ascorbic acid in the diet or as cabbage distribution) and concentration is relatively independent of the vitamin C intake (table 2).

In a report published in 1935, VILLARD demonstrated that the addition of 1 g of vitamin C in the daily ration (about 1%) failed to induce any positive or negative effects on growth performance of rabbits. Thus, it can be assumed that there is no direct toxicity of vitamin C in the rabbit. ISMAIL *et al.* (1992a) demonstrated that a daily dose of 25 mg vitamin C to breeding does reared in the hot season in Egypt (23-32°C) was able to reduce the incidence of stillbirths and kit mortality during the first week of live. This positive effect can be enhanced by the simultaneous supply of vitamin E (25 mg/day).

On the other hand, the inclusion of 1% of vitamin C increases the need for copper: with this type of supplementation a diet with 3 ppm of Cu appears copper-deficient, with symptoms similar to those described after distribution of a diet with only 2 ppm of Cu and no vitamin C addition (HUNT and CARLTON, 1965). Recent recommendations for Cu dietary level are 5 to 15 ppm minimum (MATEOS and DE BLAS, 1998). Thus, the risk of a Cu deficiency induced by an excess of vitamin C can be excluded in practical conditions. Concerning the effects of vitamin C additions, one should only bear in mind the positive effects observed in conditions of stress (25 to 30 mg vitamin C per rabbit and per day). From a practical point of view, the high fragility of this molecule must be emphasised, making necessary a protection of the vitamin if it should be included in pelleted diets (high temperature may destroy the vitamin). On the contrary, the inclusion of this water-soluble vitamin in the drinking water is very easy, but must be repeated every day.

FAT SOLUBLE VITAMINS

Vitamin A

The daily requirement of vitamin A for maximum growth was estimated at 8 µg/ kg live weight by PAYNE *et al.* (1972) and at 12 µg/kg by DONOGUE *et al* (1975). For the breeding does, it was estimated at 20 µg/kg LW by PAYNE *et al.* (1972). In addition, a daily requirement of 46 µg/ kg LW was also estimated by DONOGUE *et al* (1975) for the stabilisation of pressure of cerebrospinal liquid.

Assuming that the daily dry matter intake of a rabbit is 50 to 100 g / kg LW, according to the diet's composition and to the physiological status of the rabbit (growth, gestation, lactation, ...), these requirements are met with a feed containing 3000 IU of vitamin A/kg (1 IU vitamin A = 0.3 µg of retinol or 0.55 µg of retinyl acetate). Because of sensitivity of vitamin A to oxidation (MOGHADDAM et al. 1987), practical recommendations are higher for commercial feeds: 6 000 to 10 000 IU /kg diet.

The vitamin A supply could be efficiently met with β-carotene. The intestinal mucosa of the rabbit is able to convert β-carotene into retinol: each molecule of β-carotene is split into 2 parts, inducing finally one molecule of retinol. So 0.6 µg of β-carotene induced the liberation of 0.3µg of retinol (i.e. of one IU of vitamin A) (BONDI and SKLAN, 1984). According to OLSON and LAKSHAMAN (1970), the intestinal mucosa of the rabbit is able to transform daily 750 to 2500 µg of β-carotene per kg of rabbit live weight. After conversion, this quantity corresponds to a minimum of 375 µg of retinol which is equivalent to about 10 to 20 times the daily requirement of vitamin A of the rabbit. After this demonstration, is can be assumed that all the vitamin A required can be provided in the form of β-carotene. However, it must be emphasised that the intestinal mucosa must be "healthy" to be able to make the conversion. Effectively, the only site of conversion of β-carotene into vitamin A is the intestinal mucosa since even with high dietary β-carotene concentrations (40 to 100 mg/kg) no trace of β-carotene can be detected in the blood, the liver or the ovaries of the rabbit (KORMAN et al., 1988; BESENFELDER et al., 1993).

Toxicity of vitamin A was widely studied because of the bad habits of many breeders to add vitamin cocktails (most frequently an AD_3E cocktail) in the drinking water of their rabbits as soon as they considered that the animals suffer of "weakness" (LEBAS, 1984; CHEEKE, 1985). As mentioned above, all vitamin A needs are covered by the inclusion of only 3 000 IU of vitamin A in the diet, and most commercial feeds are supplied with about 10 000 IU of vitamin A, in addition to β-carotene contained in raw materials. For example, if a feed contains 20% of dehydrated alfalfa, this ingredient provides the form of β-carotene at the equivalent of 20 000 to 30 000 IU of vitamin A/kg (i.e. 10 time the requirement). These remarks make clear that any addition of vitamin A to a balanced commercial diet is nutritional nonsense, and it may induce toxicity problems.

Liver of a rabbit can store large quantities of vitamin A (100 000 to 200 000 IU per liver) but when saturation occurs some quantities of retinyl ester are released in the blood and toxicity signs develop. According to observations by MOGHADDAM et al. (1987), a quantity of vitamin A greater than 150 000 IU in the liver of a breeding doe (10 000 IU/kg dry matter) is a sign of intoxication. JARETT *et al.* (1988) studied levels of toxicity and deficiency through the relative concentration of retinyl palmitate and retinol in the serum. This technique is more suitable to estimate the vitamin A status of an animal than the liver vitamin A content because only a blood sample is necessary instead of having to sacrifice the rabbit. The authors have preferred to study the retinyl palmitate/retinol proportion instead of the serum concentrations, because even if both serum concentration and vitamin A liver content increase with the vitamin A daily supply, the correlation is too weak (R=0.62). According to JARETT *et al.* (1988), the lowest proportion of retinyl palmitate (6.2 ±1.8% on the total vitamin A) and the highest proportion of retinol (92.9 ± 3.5%) are observed when the vitamin A supply meets the requirement. Deficiency may be suspected when the retinyl palmitate proportion is between 8 and 21% and while the proportion of retinol is 73 to 83%. They considered that intoxication begins when the proportion of retinyl palmitate is higher than 20.4% and simultaneously to that of retinol being lower than 72.8%.

An experimental addition of 190 000 IU vitamin A / kg in the feed of pregnant does induces abortions, hydrocephalic new-borns, increase of still-born kits (hydrocephalic ones included) and high pre-weaning mortality (CHEEKE, 1984). It should be emphasised that symptoms of vitamin A toxicity are similar to those of deficiency. In the same experiment, no external sign was detectable in rabbit does, or in weaned rabbits fed with the same diet (GROBNER *et al.*, 1985).

Nevertheless, an excess of vitamin A may reduce the growth rate of weaned rabbits, as demonstrated in figure 1 (ISMAIL *et al.*, 1992b). In this work involving a small rabbit number per treatment (initially 8 rabbits at 6 weeks of age), growth rate reduction was significant above a level of 12 000 IU per rabbit per day. This corresponds to a concentration of 100 000 IU/ kg of diet (i.e. 10 time the commercial addition). To the contrary, it should be emphasized that the highest daily supply (120 000 IU / animal) is equivalent to 800 000 IU/kg (about 100 times the

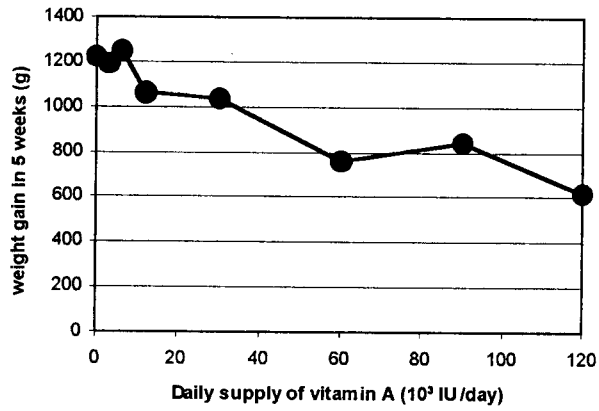

Figure 1: Weight gain over 5 weeks of rabbits fed a basic diet containing 13 200 IU of vitamin A and receiving increasing daily doses of vitamin A (oral route) according to ISMAIL et al. (1992b). The daily oral supplies were in form of retinol palmitate: 0 - 3000 - 6000 - 12000 - 30000 - 60000 or 120000 IU vitamin A.

commercial addition), but this level reduces the rabbit's growth rate to only one half of the value of the control. This demonstrates a relatively high tolerance of fattening rabbits to vitamin A excess.

Risks of vitamin A excess are not observed only in research laboratories. DEEB *et al.* (1992) observed vitamin A intoxication corresponding to the utilisation of a commercial diet containing 102 000 IU of vitamin A instead of the classical 6 000 to 10 000 IU. Signs were foetal resorptions, abortions, and high mortality at birth. The stillborn kits were abnormally developed with a high proportion of hydrocephalus, microencephalus and cleft-palate.

Another example of the bad effects of misplaced vitamin treatments was observed after periodic provision of a vitamin cocktail in the drinking water of breeding does. The distribution of a vitamin AD_3E commercial cocktail was systematically applied every 10-12 week. During the 2-4 weeks following this distribution, the proportion of stillbirth increased dramatically (figure 2). Formally it not possible to attribute this result exclusively to one of the 3 vitamins employed, but according to the symptoms (high stillbirth proportion with numerous hydrocephalus kits), an excess of vitamin A is the most plausible.

Various efforts were made during the 80's to try to demonstrate a specific need for β-carotene in the breeding doe, independently of the vitamin A requirement, as was demonstrated in the cow (KORMANN and SCHLACHTER, 1984; KORMANN *et al.*, 1988). Unfortunately, in the control diet, the vitamin A supply (20 000 to 30 000 IU/kg) was clearly higher than the classical recommendations and/or the productivity of this control group was very low (PARIGI BINI *et al.*, 1983; KORMANN and SCHLACHTER, 1984; KORMANN *et al.*, 1988).

In addition, in these publications there was no statistical analysis, and that makes the interpretation more difficult. When *a posteriori* the statistical analysis was possible, the differences don't meet the classical levels of significance. In addition, in the study of KORMANN et al. (1988), the vitamin A content of doe's liver was determined after 7 pregnancies: in the control group the vitamin A content was 186 000 IU/liver and only 54 490 IU in the β-carotene group. According to the previously mentioned data of MOGHADDAM *et al* (1987), a content of more than 150 000 IU in a liver must be considered as sign of vitamin A intoxication. Thus, it could be concluded that in the experiment of KORMANN *et al.* (1988), does of the control group were intoxicated by an excess of vitamin A (30 000 IU in the control diet). In this case, the β-carotene supplementation may be suspected to have a protective function against the vitamin A excess, at least for vitamin A storage in the liver, but the mechanism must be demonstrated.

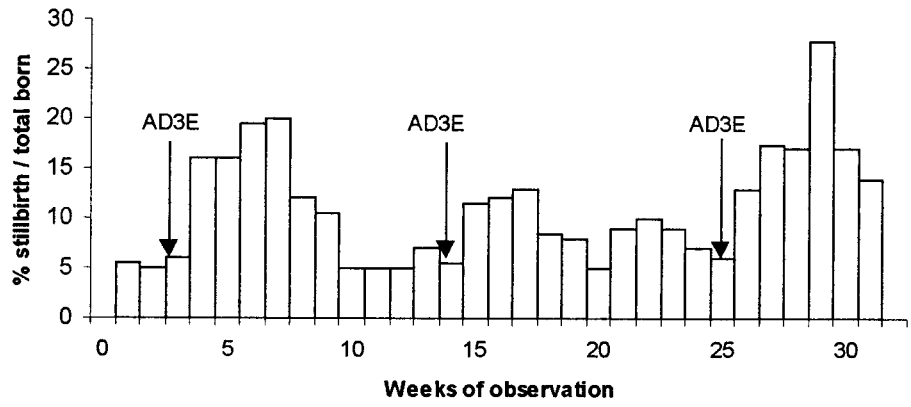

Figure 2 : Evolution during a 31 weeks period, of the stillbirth proportion in a French commercial rabbitry with 150 does (average productivity 55 young weaned per doe and per year). The AD_3E treatment was practised in the drinking water once every 3 months or all rabbits.

In a recent paper, CASTELLINI *et al.* (1992) more clearly demonstrated benefits following β-carotene addition in breeding does feeds (59 mg/kg *vs* 27 mg/kg for the control; 18 000 IU vitamin A /kg in both diets). Birth litter size was increased (9.82 *vs* 8.60 born alive), as was the percentage of fertile inseminations (78.1% *vs* 73.2%). The average litter weight at 55 days was also signi-

ficantly improved (13.72 kg vs 12.87 kg). However, some additional works are necessary to confirm these improvements and to determine the real minimum and maximum requirements of β-carotene. Effectively, in a work on the relative functions of vitamin A and β-carotene in the does reproduction physiology, BESENFELDER *et al.* (1996) did not observe any effect of a 40 ppm β-carotene supplementation, or they considered that the observed effects were strictly related to vitamin A metabolism.

Vitamin D

The daily requirement of vitamin D has not been clearly determined. However, according to results of CURRY *et al.* (1974), a value of 10-13 IU /kg live weight may be suggested (1 IU = 0.025 μg vitamin D_3). The supply was that provided to the control group in a study on vitamin D deficiency. This daily supply can be provided by a diet with 100 to 200 IU vitamin D / kg. Nevertheless, COUDERT and LEBAS (1982) have demonstrated that a concentration of 300 IU /kg remains insufficient. In practice, diets with 600 to 1000 IU/kg of vitamin D have been proven to provide sufficient amounts of vitamin D for growing or breeding rabbits (BOURDEAU *et al.*, 1986; DE PALO *et al.*, 1988). In case of vitamin D deficiency, intestinal absorption of calcium and phosphorus are not modified, but renal excretion of calcium is reduced. This induces a better calcium raw balance in vitamin D deficient rabbits than in normal ones (BOURDEAU *et al.*, 1986).

As for vitamin A, the main practical problems encountered are related to vitamin D excess. One of the reasons is the uncontrolled utilisation of vitamin AD_3E or AD_3 cocktails as mentioned above. Another reason is the frequent excessive vitamin D addition in the commercial diets. Symptoms of vitamin D excess (i.e. calcification of soft tissues, mainly aorta, kidneys, ...) were observed with a diet containing 3250 IU vitamin D/kg (LÖLIGER and VOGT, 1980). The risk of excessive calcification increases with the calcium level in the diet above the rabbits requirements (6 to 12 g/kg according to MATEOS and DE BLAS, 1998). It should be emphasize that in the work of LÖLIGER and VOGT (1980), rabbit live weight gain during the 10 experimental weeks was significantly higher with 6250 IU vitamin D / kg diet than with lower vitamin D levels (1250 or 3250 IU/kg); but simultaneously, first signs of aorta and kidneys calcification were visible in this group after 4 weeks and more clearly after 8 weeks.

The administration to pregnant does of 10 000 IU of vitamin D_3 per day between the 26th and the 28th day of pregnancy, increased the proportion of dead foetuses observed on the 29th day, from 2.8% in the control, to 17.9% in the experimental group (KUBOTA *et al.*, 1982). Quite similar results were observed by CHAN *et al.* (1979) after injection of 10 000 to 100 000 IU vitamin D_2 every other day during the first 28 days of gestation of rabbit does. Lastly, significant calcification of the aorta wall of adult rabbits was observed after utilisation of a feed containing 5 000 IU vitamin D/kg (KAMPHUES *et al.*, 1986). These different experimental results demonstrate that any additional distribution of vitamin D (in drinking water or in solid feed) may provoke deleterious effects without improvement of productivity. For commercial feeds, the vitamin D supplementation should be between 800 and 1 000 IU / kg and never higher than 2 000 IU.

Problems of anorexia, intense thirst, ataxia and mortality were observed in rabbits in a Canadian rabbitry fed with a diet containing 7 230 IU of vitamin D/kg (STEVENSON *et al.*, 1976). In dead rabbits, small and medium arteries were calcified, as were aorta, hearth, adrenals, and spleen. In addition, the structure and mineralization of bones were altered. Similar problems were observed in USA in relation to the utilisation of a feed containing 13 200 IU of vitamin D. Nevertheless, it is necessary to be cautious after a simple observation of calcified aortas in adult rabbits from *post mortem* examinations (ZIMMERMAN *et al.*, 1990). Effectively, NOUAILLE *et al.* (1994) have observed aorta calcification in 40% of females older than 2 years, despite the utilisation of balanced diets (1 000 IU vitamin D / kg, Ca and P within the recommendation limits). Proportion of does with aorta calcification was only 1.5% for animals younger than one year and 28% for those between 1 and 2 years of age.

Finally, according to RAMBECK *et al.* (1990), in the rabbit, unlike the observations made in the rat, vitamin D_3 ($1,25(OH)_2D_3$) is slightly more toxic (inducing soft tissue calcification) than vitamin D_2, and the metabolites 1□(OH)D_3 and 1□(OH)D_2 are half toxic as the corresponding vitamins. The effects on calcification of vitamin D_3 and of its palmitic ester are similar (RAMBECK *et al.*, 1981). According to the observations of HENDERSON and EASON (2000), the LD_{50} of vitamin D_3 (lethal dose for 50% of the rabbits) corresponds to the ingestion of 176 000 IU /kg live weight (4,4 mg vitamin D_3 /kg LW) and any dose higher than 600 000 (15 mg /kg LW) is lethal for all rabbits.

Vitamin E

The daily requirement of vitamin E (dl-α-tocopherol) ranges between 0.32 and 1.4 mg/kg live weight according to HUNT and HARRINGTON (1974). The d isomer seems more efficient than the l isomer because of a faster elimination of d-α-tocopherol (FITCH and DIEHL, 1965). This daily requirement can be supplied by a diet containing a minimum of 25 mg of vitamin E /kg. According to RINGLER and ABRAMS (1971), a diet with only 16.8 mg vitamin E /kg is able to provoke deficiency symptoms. Thus, a level of 40 to

50 mg/kg diet could be recommended under practical conditions.

The most common symptom of vitamin E deficiency is muscular dystrophy (JOHANSSEN, 1972). The corresponding external signs are locomotion incoordination. But, vitamin E deficiency may also provoke sudden death without any previous signs if the cardiac muscle is first damaged (LOPEZ FUENTES, 1989). These authors observed high mortality in Spanish commercial rabbitries where a diet low in vitamin E was utilised. This abnormally low level of vitamin E may be related to the utilisation of alfalfa hay that has a lower vitamin E content (40 mg/kg in normal situation) than dehydrated alfalfa (120 mg/kg according to the INRA (1989) tables and 200 mg /kg according to CHEEKE, 1987). From a general point of view, dehydrated alfalfa seems to be a good source of vitamin E. Effectively, according to CHEEKE (1987) its vitamin E content is 10 to 20 times higher than the vitamin E of any other common feed ingredient. Effectively, in a diet with 25% dehydrated alfalfa, this ingredient supplies 30 to 50 mg vitamin E per kg of diet that covers the rabbits requirements. The utilisation of a high proportion of dehydrated alfalfa in French commercial pelleted feeds (20 to 30%) can justify a very variable supplementation of these diets with vitamin E (addition of 0 to 50 mg /kg) without any vitamin E deficiency symptoms (LEBAS *et al.*, 1981). But the Spanish observations mentioned above are sufficient to note the deficiency risk if alfalfa does not contain the expected concentration of vitamin E (level of this vitamin is not a quality criterion in commercial transactions - only protein, β-carotene and even fibre levels are taken in consideration).

YAMINI and STEIN (1989) have observed in some American breeding units the classical symptoms described above (muscular dystrophy, hearth damage, ...), but vitamin E deficiency was also associated with reproduction problems : low fertility, high proportion of abortion, increased stillbirth rate and high *post partum* mortality of kits. The situation of these commercial units was corrected by supplying wheat germ oil, one natural source of vitamin E. Male reproduction is also affected by vitamin E deficiency, mainly during the hot season (EL MASRY *et al.*, 1994). The inclusion of 40 mg vitamin E /kg diet combined with selenium may reduce the effect of heat stress on sperm concentration and serum testosterone level. Finally, it must be emphasised that in the opposite to the situation of vitamin A and D, toxicity of vitamin E has never been described (HUNT and HARRINGTON, 1974).

Because of the strong tolerance of rabbits to high vitamin E dietary levels, and because of the antioxidant activity of the α-tocopherol molecule, many experiments have been conducted with the objective of increasing the vitamin E concentration in the animal muscle tissue and then in rabbit meat. Such an increase is suitable to improve meat shelf-life by reduction of lipid oxidation activity (BERNARDINI *et al.*, 1996; CASTELLINI *et al.*, 1998). Only VERSCHUREN *et al.* (1990) brings into question the real efficiency of vitamin E (tested levels up to 500 mg/kg of diet) to exert this antioxidant activity in the case of the *n*-3 long chain fatty acids. On the contrary, BERNARDINI *et al.* (1996) and CASTELLINI *et al.* (1998) have effectively observed a reduction of the oxidation speed of raw and cooked meat of rabbits that were fed a diet with 200 mg/kg vitamin E (only 50 mg in the control). This reduction of peroxidation was shown by CHAN *et al.* (1983) to be due to the reduction of hepatic NADPH oxidase when rabbits were nourished with a diet containing 200 mg vitamin E /kg. Apart from this real antioxidant activity of high levels of vitamin E, no consequences were observed on growth rate, feed efficiency or slaughter rate.

The antioxidant activity of vitamin E was also tested as a hypo-cholesterolemic agent. WILSON *et al.* (1978) worked with a vitamin E supplementation of 1% (10 000 mg/kg). They have effectively observed a reduction of the cholesterol level in the serum, but they have also demonstrated the strong tolerance of rabbits to such a massive administration of vitamin E.

Vitamin K

Vitamin K is known for its role in the synthesis of blood coagulation factors. Some new roles of vitamin K have now been demonstrated (MATEOS and DE BLAS, 1998). This fat-soluble vitamin is synthesised in large quantities by the digestive flora, and in absence of digestive problems , these quantities are sufficient to cover all the vitamin K requirements. Nevertheless, several years ago we unintentionally provoked a vitamin K deficiency (LEBAS, 1968 - unpublished data). At that time, we worked on doe nutrition with semi-purified diets based on soya oil cake, pure starch and cellulose + minerals and classical vitamin AD_3E supplementation. The deficiency develops in absence of any digestive disorders . It was characterised by a high abortion rate as described by MOORE et al. (1942) but above all by a dramatic decrease of the blood speed of coagulation of the newborn kits. All symptoms disappear after addition of 2 ppm of vitamin K in the diet.

Considering the high vitamin K content of most forages, and especially of dehydrated alfalfa (20-35 ppm), the risk of vitamin K deficiency can be ignored for commercial feeds. Nevertheless, MATEOS and DE BLAS (1998) advised to include 2 ppm of vitamin K as a precaution, particularly when rabbits may suffer from sub-clinical coccidiosis, frequent sulfa-drug treatments or prolonged antibiotic treatments.

Table 3 : Recommendations for vitamin levels in commercial balanced rabbit feeds

Vitamins (units / kg or ppm)	Advised Supplementation	Supplementation used without problems (non-purified diets)		Levels known to provoke problems	
		Mini	Maxi	Deficiency	Toxicity
Vitamin A (retinol IU)	**10 000** (1)	6 000	20 000	2 000	100 000
Vitamin D (IU)	**1 000**	600	2 000	300	3 500
Vitamin E (ppm)	**50**	25	10 000	17	(2)
Vitamin K (ppm)	**2**	0	5	0	(2)
Vit. B_1 (thiamin, ppm)	**2** (3)	0	(5)	-	(5)
Vit. B_2 (riboflavin, ppm)	**6** (3)	0	(5)	-	(5)
Niacin (vit. PP, ppm)	**50** (3)	0	(5)	-	(5)
Pantothenic acid (ppm)	**20** (3)	0	(5)	-	(5)
Vit B_6 (pyridoxine, ppm)	**2** (3)	0	(5)	-	(5)
Folic acid (ppm)	**5** (3)	0	(5)		(5)
Vit. B_{12} (ppm)	**0.01** (3)	0	(5)	-	(5)
Biotine (ppm)	**0.10 (3)**	0	(5)	-	(5)
Choline (ppm)	**200** (3)	0	(5)	-	(5)
Vitamin C (ppm)	**250** (4)	0	10 000	-	(2)

(1) may by supplied by 30 ppm of β-carotene. No toxicity of β-carotene
(2) no known toxicity, even with a massive supply
(3)supplementation advisable in case of risk of digestive trouble (post-weaning, ...)
(4)supplementation advisable in situation of stress (heat, ...) in a protected form, and
(5) no information available on risks of toxicity in relation to massive supplementation of B-complex vitamins, but it is improbable because of the very low capacity of storage of these vitamins in the rabbit body

PRACTICAL RECOMMENDATION AND RISKS

In the table 3, practical recommendation are made for commercial diets. Because of the small number of publications, recommendations are made without consideration of physiological status, and even if we knew that requirements vary widely, depending on rabbit's physiological status . In the table, we have included the highest and lowest concentration of vitamins known for their utilisation without apparent (detected) problems. We have also included levels known to provoke deficiency or toxicity symptoms.

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