Artykuł przeglądowy *Review*

Factors affecting vitamin E level in ruminant tissues

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Summary

Literature was surveyed to clarify the role of factors affecting vitamin E deposition in animal tissues and findings were compared. Special attention was paid to ruminants because of the rumen factor having a different environment for nutrients and being absent in monogastric animals. A hypothesis is proposed that even together with good quality grass high intakes of vitamin E may not protect the animal from a depletion of this vitamin in its tissues. Special attention must be given to diet ingredients and animal health status when vitamin E is used in ruminant diets.

Keywords: vitamin E, ruminant

Successful animal production requires that farm animals be provided daily with food that is appropriate to their age and requirements. Absence or deficiency of nutrients as well as one or more vitamins results in metabolic disorders that manifest themselves as reduced output, growth retardation. Dietary deficiencies have now become rare, however without vitamin E in animal feeds they would become apparent in the form of disturbances of growth and fertility. In addition to the known roles of vitamin E as a nutrient in reproduction, animal health, its role in product quality has been recognised. Antioxidant vitamins C, E and beta carotene may play an expanded role in domestic food animals when fed at levels above NRC requirements by enhancing the immune system and optimising animal product quality (19). There is also an increased requirement for dietary tocopherols as dietary unsaturated fat is increased. However, despite the high level of supplementation of vitamin E to diets, lower concentrations of this vitamin in tissue and milk was observed (9, 14) when diets supplemented unsaturated fats in ruminants.

The aim of this article is to summarise main effects of supplemental vitamin E animal tissue concentrations and to review studies on vitamin E in tissue concentrations special attention to ruminants but in some cases for monogastric animals reviewed.

Diet

Increasing the level of the readily available carbohydrates aimed to increase microbial action in the rumen induces subsequently vitamin E destruction. If microbial action in the rumen results in vitamin E disappearance the increased fermentation rate can be expected to accelerate this disappearance (2). Alderson et al. (2) showed that the amount of α-tocopherol disappearing is proportional to the content of dietary concentrates reaching 50% in an 80% corn diet fed to sheep. Similarly, Shin and Owens (43), using adult steers fed a rolled corn-cottonseed hull finishing diet, found that 39 to 52% (depending on the form, 38% for free form and 16% for dl-α-tocopherol acetate) of supplemented vitamin E disappeared before reaching the duodenum. Furthermore, the rate and magnitude of appearance of orally administrated tocopherol in the plasma of ruminants was substantially lower than that obtained with intramuscular injections, suggesting that intestinal absorption of this compound is relatively poor (8). Likewise, Alderson et al. (2), Hidiroglou and Jenkins (23) and Hidiroglou et al. (24) reported higher tissue and plasma levels when radiolabeled α-tocopherol was dosed into the duodenum than when it was dosed into the rumen. Indirectly supporting this hypothesis, Rode et al. (40), quantified vitamin A disappearance in ruminal fluid from cattle fed concentrate, hay or straw diets. These authors estimated effective ruminal degradation of vitamin A for these diets of 67, 16 and 19%, respectively. Vitamins A and E are closely related in that they are both fat-soluble, have similar distribution in the fatty tissues of the body, and protect the host from oxidative injury (30). For these reasons, many researchers believe that Vit A + E will undergo similar reactions in the rumen. But these two vitamins actually behave quite differently. In contrast to vitamin E, vitamin A has four double bonds in its side chain, which are susceptible to reduction in the anaerobic environment of the rumen because double bonds are targets for biohydrogenation by a number of

ruminal microbes (22). In contrast to vitamin A, the double bonds of tocopherols are within an aromatic ring. Aromatic rings are not readily biohydrogenated or degraded anaerobically under prevailing conditions and retention times in cattle feeding high-grain diets (10).

In contrast to the above, Astrup et al. (4) found no significant disappearance of dl-α-tocopherol during a 24 h *in vitro* incubation period after feeding fasted sheep with an alfalfa chaff : oats (1 : 1) diet. These workers also used rumen contents from animal fed low quality forage. The microflora from an animal fed a high-concentrate diet is sufficiently different metabolically from that of an animal fed a high-forage diet to support the purported ruminal destruction of vitamin E (28, 40). Leedle et al. (29) also reported that vitamin E (α-tocopherol acetate) was not microbially degraded, during 24 hour *in vitro* incubation in ruminal contents (pH 4.9-5.7) from concentrate fed animals. These authors concluded that the results documenting disappearance are not due to degradation but to inadequate extraction methods or prediction of vitamin E transport, or vitamin E oxidation during collection of digesta due to lack of adequate protection. Chikunya et al. (11) also found that vitamin E (as dl-α-tocopherol acetate) did not disappear in the rumen and passed through the duodenum in lambs fed different α-3 PUFA (polyunsaturated fatty acids) sources.

Level of vitamin E in diet

Some researchers reported that the intestinal absorption of vitamin E is relatively inefficient, particularly for large doses in rats (31, 48). Both deficiency and excess of dietary vitamin E decreased the levels of lipids and cholesterol in the plasma of rats (48). The decrease in plasma total lipids was greater than that of plasma cholesterol in rats fed excess vitamin E, suggesting that other plasma lipid components, such as triacylglycerols or phospholipids might also be affected. Goff and Stabel (20) observed low concentrations of α-tocopherol when plasma lipids were low. Tocopherol and β-carotene are transported in the plasma via lipoproteins (5) and a decreased concentration of lipoproteins may limit the capacity of plasma for these nutrients. Reported values by Christie (12) for plasma total lipids in sheep are between 0.163-0.224 g per 100 ml plasma. Increasing the concentration of lipids in plasma may increase the concentration of circulating α-tocopherol (47). Increasing dietary levels of vitamin E also might increase both the intestinal absorption and liver storage of vitamin A in rats (27, 48). Early work by Dicks et al. (15) demonstrated that vitamin A can depress vitamin E utilisation in ruminants. Yang and Desai (48) concluded that further increases in dietary vitamin E only increased retention of vitamin A, which occurred only at certain critical levels of vitamin A and vitamin E. On the other hand, Hino et al. (26) showed that high concentrations of vitamin E inhibited bacterial growth in the rumen. For example, when 10 mg/L vitamin E were added to 100 mg/L of safflower oil, bacterial growth significantly increased (from 35 mg/L to 45 mg/L). But, when 40 mg/L vitamin E were added, bacterial growth significantly decreased (to 15 mg/L). However, the combination of β-carotene and α-tocopherol (each 5 mg/L) exerted partially additive effects (to 55 mg/L). The authors suggested that instead of the use of high levels of vitamin E in ruminant diets, addition of β-carotene and α-tocopherol to the ruminant diets may have beneficial effects on the growth of rumen microbes and fibre digestion especially when fats are supplemented.

Form of vitamin E

Because the free form is easily oxidised, more stable forms such as acetate and succinate esters have been synthesised (32). The formation of esters to stabilise vitamin E products against oxidation is necessary for some types of products. Dudin and Dvinskaya (17) reported that when 1.5 g α-tocopherol acetate is introduced into the rumen of 6 months old heifers, α-tocopherol acetate was not hydrolysed in the stomach and entered the small intestine unchanged. These authors recommended that the ratio of free to esterified forms of α-tocopherol in the diet should be considered in estimating vitamin E requirement. The need to convert ester forms back to α-tocopherol by gastrointestinal tract esterases before absorption (6, 42) probably means that only under optimal conditions are the ester forms absorbed as well as the alcohol forms. Several studies have indicated that there may not be adequate intestinal esterase activity in ruminants to maximise the absorption of tocopheryl esters (25, 41). Roquet et al. (41) also pointed out that pancreatic lipase which converts the acetylated forms to free forms acts only on previously emulsified fats. The fact that acetylated forms of vitamin E require these additional steps may explain why esterified forms of vitamin E given directly into the duodenum are so poorly utilised. Combs (13) indicated the importance of emulsification of micelles for α-tocopherol absorption. The formulation of micellized tocopherol supplement, such as that used by Pumfrey et al. (35), could be expected to enhance absorption.

Polyunsaturated fatty acid effect

Ruminant meat and milk products can be made more nutritionally desirable by increasing PUFA, adjusting the n-3/n-6 PUFA value and controlling trans fatty acids and conjugated linoleic acid (CLA) (14, 45). This is performed by adding PUFA sources directly or after processes to animal diets (44). Amounts of PUFAs in the diet affect ruminants vitamin E requirements (37). However, medium-chain triglycerides enhance the absorption process, whereas retinoic acid and long chain PUFAs may reduce the absorption of α-tocopherol. Long chain PUFAs may also enhance the oxida-

tion of α-tocopherol *in vivo* (16). When PUFA were offered together with α-tocopherol, only half of the α-tocopherol was recovered from the rats given C18 : 2 *n*-6, compared to rats offered C18 : 1 *n*-9 (21). Feeding cod liver oil instead of lard decreased tissue α-tocopherol concentration and accelerated vitamin E deficiency symptoms in rats (21). Gallo-Torres (18) also showed that increase of the linoleic acid content in the emulsion led to a greater decrease in tocopherol absorption of tocopherol to such a point that only 7% was absorbed when the emulsion contained 32% of the fatty acids whilst a higher percentage of glyceride was found to promote a correspondingly higher absorption of the vitamin E. These results show that the presence of the lipid fraction does reduce significantly the amount of vitamin E that can pass through the intestinal barrier. Gallo-Torres (18) concluded that the increase in the lymphatic appearance of vitamin E when emulsion contains a glyceride rather than a free fatty acid, was perhaps related to enhanced chylomicron formation. It is possible, therefore, that the synthesis of lipoproteins is a rate-limiting factor in the absorption and transport of vitamin E. An alternative explanation, which may be more important in ruminants, is that the presence of monoacylglycerols in the intestinal lumen enhances emulcification and vitamin E absorption. Their absence from the ruminant intestine could be a cause of poor vitamin E absorption. Alderson et al. (2) speculated that lower vitamin E absorption with increased PUFA may be due to unsaturated fatty acids having greater space occupying requirements on VLDL than saturated fats.

In the work of of Chikunya et al. (11) the concentration of vitamin E in plasma in the current study decreased as both the amount and level of polyunsaturation in the diet increased with Megalac (containing palm oil) > Linseed > Linseed plus fish oil mixture. It is well established that ruminant diets need to be supplemented such that adequate vitamin E is supplied in relation to their content of polyunsaturated fatty acids (36). Putnam and Comben (36) suggested that 3 mg (3 IU) of vitamin E should be supplied per each gram of added PUFA. However, the susceptibility of PUFA's to peroxidation is also related to the number of double bonds in the long chain PUFA molecules (33).

Se and gluatation peroxidase

In some cases despite the low vitamin E status (both plasma and muscle) animals cannot express deficiency signs (14). It could be that other defence mechanisms operating *in vivo* prevent or minimise tissue damage thus preventing clinical deficiency signs. One such defence mechanism involves selenium as part of the glutathione peroxidase and glutathione lipid hydroperoxide peroxidase. The level of glutathione peroxidase in tissues depends on the concentration of selenium in the diet (39) whereas feeding diets low in α-tocopherol had no effect on level of glutathione peroxidase activities (46). When selenium levels in the diet averaged 1.0 mg/kg DM compared to the recommended levels of 0.3 mg/kg DM (3), high selenium levels may have increased the level of glutathione peroxidase enzyme in tissues (14). According to Brzezinska (7) if the selenium content in feed is less than 0.12 ppm, the Vitamin E supplementation for cows may not be effective.

Animal age

Rumen development affects the degree of PUFA hydrogenation and thus the levels of vitamin E requirements (37). Moreover, in very young ruminants when the rumen is not fully functioning vitamin E destruction by fermentation is not as significant as in the adult ruminant (34).

Health status

Vitamin E deficient ruminant animals absorb 50--75% of their tocopherol intake whereas normal animals absorb 20-30% of their tocopherol intake. Animals receiving an excess of vitamin E absorb only 1-5 of their tocopherol intake (37). Gastrointestinal, digestive or absorptive disturbances affect absorption of tocopherol. Lipid malabsorption is also associated to the degree of tocopherol absorption (1, 13). In addition vitamin E deficiency may be caused as a defect in the incorporation of α-tocopherol in lipoprotein secreted by the liver (16). Other factors include the inherent or genetically predetermined degree of unsaturation in the cell membrane and the inherent ability of the subcellular membrane, under attack, to maintain high concentrations of peroxidation antagonists, such as vitamin E, GSHPx, catalase, etc. within the cell (38).

Conclusion

When all the observations discussed above are taken into account, it becomes evident that the digestion-absorption of dietary vitamin E is a complex biochemical process requiring the normal functioning rumen and of the small intestine as well as adequate secretion of the bile and pancreatic lipase. Dysfunction of one of these negatively affects of absorption and cause deficiency of this vitamin in animal and also affects distribution of vitamin E in tissues and results in the loss of nutritionally desirable PUFA and a reduction in meat quality. These results show the importance of muscle vitamin E concentration for animal health and meat quality.

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ASTORGA R., ARENAS A., TARRADAS C., MOZOS E., ZAFRA R., PÉREZ J.: Ognisko nadostrej posocznicowej postaci salmonellozy u koni związane z równoczesnym zakażeniem *Salmonella Enteritidis* i *Mucor sp.* (Outbreak of peracute septicaemic salmonellosis in horses associated with concurrent *Salmonella Enteritidis* and *Mucor* species infection). Vet. Rec. 155, 240-242, 2004 (8)

W stadninie koni w Sewilli liczącej 45 zwierząt w październiku u 9 koni wystąpiła gorączka (41°C), utrata łaknienia, biegunka, bóle brzucha, niechęć do poruszania się i powiększenie obwodowych węzłów chłonnych. Nie uzyskano polepszenia stanu zdrowia po zastosowaniu interprimu w dawce 30 mg/kg/dzień i cefaloksyny w dawce 10 mg/kg/dzień. Wszystkie chore konie padły w ciągu 2-4 dni. Badanie zwłok 18-miesięcznego źrebięcia na Wydziale Weterynaryjnym w Kordobie wykazało owrzodzenie okrężnicy i jelita ślepego. Śluzówka jelit była przekrwiona i obrzękła, a wybroczyny występowały pod otrzewną, opłucną, torebką śledziony i w gardzieli, na osierdziu i wsierdziu. Płuca były przekrwione i obrzękłe. W preparatach sporządzonych z oskrzeli występowały strzępki grzybni *Mucor sp.* i bakterie Gram-ujemne, które zidentyfikowano jako *Salmonella Enteritidis* (profil 9,12:g.m) typ fagowy 21. Wyizolowany szczep bakterii był wrażliwy na ampicylinę, tetracyklinę, trimetoprim-sulfametoksazol, sulfonamidy, enrofloksacynę, ceftiofur i florofenikol.

G.