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The Rationale behind the Induction of Hypovitaminosis A in Feedlot Cattle and the Clinical Consequences

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Summary

The rationale for precluding vitamin A or its precursor, β-carotene, from the diet of feedlot cattle is based on specific market requirements. β-carotene accumulates in fat, making fat undesirably yellow and, in addition, there is a possible link between vitamin A deficiency and the increased marbling of meat: a characteristic considered desirable by the Japanese market. Evidence from *in vitro* studies suggests retinoic acid (a derivative of vitamin A) has a regulatory effect on cell differentiation and it has been shown that low levels cause differentiation and proliferation of fat cells. It is also involved as a messenger in gene transcription, in particular, for growth hormone.

Under Australian climatic conditions, a feeding regime which precluded vitamin A or its precursor, β-carotene, was shown to have serious consequences. A combined deficiency of vitamin A and E was diagnosed in finisher cattle in a 12,000 head feedlot producing beef for the Japanese market. The clinical signs, seen in cattle between 220 and 300 days on feed, ranged from poor vision, lameness, subcutaneous and tissue oedema, to heat intolerance, recumbency and death. The clinical condition was only observed during the warm to hot months.

Following the commencement of oral supplementation of vitamins A and E, the clinical condition disappeared and serum and liver vitamin A and vitamin E levels rose to clinically acceptable levels.

Introduction

Vitamins A and E have long been recognised as necessary supplements to maintain the health of feedlot cattle. These cattle require vitamin A when liver stores become depleted. Unlike cattle grazing fresh green feed, feedlot cattle have a limited intake of β-carotene, the precursor of vitamin A, and hence a limited ability to produce vitamin A. Their requirements for the vitamin are much greater than those of other cattle because of the faster growth rate associated with feedlot rations. Supplementary vitamin E is required also in their diet because the levels deteriorate in stored feed, and because the feed contains components such as polyunsaturated fats which increase vitamin E requirements.

In recent years, particularly in Japan, vitamin A has been intentionally left out of feedlot rations because of the belief that this enhanced the marbling of beef. The term marbling refers to the deposition of adipose tissue in muscle, a characteristic considered desirable by the Japanese market. Recent scientific research has supported this belief. Numerous studies in cell culture (Gudas *et al* 1994) and in rats (Haq and Chytil,1992), show that retinoic acid can inhibit the differentiation of fibroblasts

into adipocytes and conversely that low levels of retinoic acid enhance the differentiation into adipocytes. In support of these findings, studies in Wagyu and Wagyu cross Holstein cattle, demonstrated that serum retinol concentrations were negatively correlated with beef marbling performance (Torii *et al* 1996). Kitagawa (1992) noted that these serum concentrations could also be associated with undesirable effects on carcass quality: lowered body weight and oedema of the muscle.

Background

The feedlot involved in this investigation caters for the Japanese market and fattens steers for approximately 300 days prior to slaughter. The steers, originally from grazing properties, are purchased at 12 to 18 months of age with an average weight of 400 kg. By day 200 in the feedlot they weigh approximately 700 kg. The diet is based on grain and corn silage with the percentage of grain being increased in relation to the time on feed. The grains used, sorghum, barley and wheat depend on seasonal availability. At the time of the investigation other feed additives included cottonseed meal, bagasse, sunflower kernels, oilpalm kernel husks, brewery water, wheat bran and lucerne cubes. No fresh green feed was included in the diet. In the last 100 days, the diet is formulated to encourage meat flavour and marbling but there is little weight gain. There was no oral supplementation with vitamins A and E although at the time of entry each animal was injected with 2 ml A.D.E. (Heriot) which contains 1.000,000 IU Vitamin A; 150,000 IU Vitamin D_3 and 100 IU Vitamin E.

Over two consecutive summers a clinical illness was observed in finisher cattle in the 12,000 head feedlot. It was estimated that approximately 2,000 cattle were affected to varying degrees at any one time (i.e. cattle between 220 and 300 days in the feedlot).

Clinical signs and epidemiology

The clinical condition became obvious in the warmer months of the year from October until March. Animals were noticed to be suffering from clear ocular and nasal discharges, swollen hind legs, varying degrees of lameness and inco-ordination, difficulty in rising particularly in the hind legs, recumbency, exophthalmus, 'glazed' bluish appearance to the eyes, poor adaptation to dull light with pupils more dilated than normal and occasional cases of complete blindness with no pupillary light reflex. In advanced cases the swelling or oedema in the hind legs progressed to include oedema of all four legs, and extended to the shoulder and under the jaw. A number of affected animals were also seen to have corneal ulcers, possibly as a result of injuries incurred through poor vision. Affected animals also showed a poor tolerance of hot weather. Their body temperatures were above normal (39.5°C - 41°C) in the middle of the day (ambient temperature 23.8°C and no wind) when compared with unaffected cattle. This was associated with increased respiration, drooling of saliva and occasionally panting. When observed in the cool evening these same animals showed no evidence of hyperthermia. Deaths of clinically affected animals occurred during hot weather and during prolonged transportation.

Gross pathological findings

The most obvious finding was massive subcutaneous and intramuscular oedema involving the hindlegs, forelegs, shoulders, ileopsoas lumbar region and overlying the ribs. Oedema fluid was particularly obvious in the fascial plains between muscle bundles. This fluid was clear, yellow tinged and proteinaceous. Occassional ecchymotic haemorrhages were observed in muscle. The subcutaneous tissues of the lower legs were oedematous and composed of what appeared to be a massive proliferation of small blood vessels. The sciatic nerves were markedly distended with oedema fluid. Joints, particularly hock joints were swollen and contained clear yellow proteinaceous fluid. The synovial membranes appeared to be excessively vascular and inflamed. The lungs were mildly congested and small vascular tags were visible on the pleural surface. There was excess but not voluminous yellow tinged pleural and pericardial fluid. The skeletal muscles were heavily infiltrated with fat.

Histopathological findings

Pathological findings consistent with vitamin A deficiency included squamous metaplasia of the parotid salivary duct and mild papilloedema of the optic nerve. In line with the gross pathological findings, histopathologically oedema occurred in subcutaneous tissue, within muscle bundles and within nerve bundles. An apparent proliferation of small blood vessels and microangiopathy were identified in association with and probably as a cause of the oedema. This microangiopathy was identified in blood vessels in subcutaneous tissue, muscle and on the pleural surface of the lungs, diaphragm and chest wall. Microscopically there was damage to endothelial cells lining small blood vessels with subsequent leakage of fluid and protein into surrounding tissues. Blood vessel walls were oedematous, endothelial cells were swollen, the lumen of many vessels was obliterated and thrombosis was present. In some vessels hyaline degeneration was observed with a marked inflammatory response.

Biochemical findings

From severely affected animals both serum and liver levels of vitamin A and E were below normal (Refer to Table 1). Serum levels of both vitamins were measured in relation to "days on feed". Both levels dropped with time, with vitamin A dropping below its critically low level at approximately 180 days and vitamin E not dropping below its critically low level, except in animals with severe clinical signs. That is, vitamin A levels appeared to become critically depleted first, and vitamin E levels only became critically depleted in severely ill animals. Creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) values were elevated in severely affected animals. Glutathione peroxidase levels were normal.

Table 1. Serum and liver vitamin A and E levels in 2 animals with severe clinical signs.

	Animal 1	**Animal 2**	**Normal reference values**
Serum vitamin A (μm/l)	0.1	0.0	>0.9
Serum vitamin E (μm/l)	1.9	2.5	>4.6
Liver vitamin A (μm/kg)	2.4	0.0	>50
Liver vitamin E (μm/kg)	10.1	1.9	21-102

Response to treatment

Following the commencement of oral supplementation at a rate of 40,000 IU vitamin A, 11.2 IU vitamin E per head per day, the clinical condition disappeared and serum vitamin A levels rose above the critical level. The vitamin E level has since been increased to 100-500 IU per head per day.

Discussion

Some of the essential functions of vitamin A have been appreciated for a long time. In fact, vitamin A was the first vitamin recognised through its role in growth and eye function. It was shown to be essential in retinal pigment, reproduction, and the structure and function of epithelial cells. In recent years studies have demonstrated the hormone-like actions of retinoic acid which is the principal metabolite of retinol. Retinoic acid regulates a large number of genes which in turn control functions as diverse as embryonic development, the functions of epithelial cells, growth factors and many aspects of cellular metabolism. Specific nuclear receptor proteins for retinoic acid have been identified (Ross and Ternus, 1993). It is also involved as a messenger in gene transcription, in particular for growth hormone (Gudas *et al* 1994).

Retinoic acid has been shown to induce differentiation of many cells. In contrast, numerous *in vitro* studies have shown that high levels of retinoic acid inhibit the differentiation of adipocytes and conversely low levels enhance differentiation. Not only is differentiation inhibited by high levels of retinoic acid, there is a decrease in the activity of lipogenic enzymes and in levels of fatty acid binding protein. Retinoic acid interacts with nuclear retinoic acid receptors (RARs) which in turn regulate expression of specific genes (Haq *et al*,1991; Haq and Chytil, 1992). The majority of research publications in this field agree that retinoic acid can inhibit the differentiation of fibroblasts into adipocytes although Safonova *et al* (1994) showed that fatty acids and retinoids can act synergistically to stimulate adipose cell differentiation.

Meat marbling is either due to an increase in adipocyte numbers (hyperplasia) or to an increase in adipocyte volume (hypertrophy). Unlike fat at other body sites, intramuscular fat can undergo hyperplasia in adult animals and it has been demonstrated that this hyperplasia is important in the marbling of beef (Torii *et al* 1996). In support of the laboratory studies of the effect of retinoic acid on fat cell differentiation, on-farm studies in Wagyu and Wagyu cross Holstein cattle, have shown a negative correlation between serum retinol concentrations and beef marbling performance (Torii *et al* 1996). It has yet to be ascertained whether this is true for other breeds of cattle.

Although there is evidence that meat marbling may be enhanced by inducing low serum levels of vitamin A, this practice runs the risk of inducing the severe clinical manifestations of vitamin A deficiency. The clinical signs associated with vitamin A deficiency have been well documented and vary in relation to the age of the animal. In calves, the arachnoid villi (the principal site for drainage of cerebrospinal fluid) and the retina are the tissues most sensitive to vitamin A deficiency (Divers *et al*,1986), resulting in night blindness, complete blindness, increased cerebrospinal fluid pressure, papilloedema, and convulsions. Also bone growth is affected in growing animals (Davis *et al*, 1970, and Van der Lugt *et al*, 1989) resulting in skeletal abnormalities which in turn may affect central nervous system function. Mature animals show no bone abnormalities, but have loss of retinal pigment, retinal degeneration and squamous metaplasia of epithelial cells with resultant loss of function of epithelial surfaces.

In this report, the affected cattle exhibited ocular changes and squamous metaplasia of epithelial cells. In addition many of the affected animals exhibited clinical manifestations that have only occassionally been associated with vitamin A deficieny. These included severe subcutaneous and tissue oedema and an inability to cope with heat stress.

Oedema in vitamin A deficient cattle was first reported in 1941 (Moore) and then in 1947 (Madsen and Earle) but in the many subsequent publications on vitamin A deficiency, oedema generally has not been reported as a clinical sign. More recently Japanese researchers have observed oedema in vitamin A deficient cattle and refer to this condition as “zulu” (Okuda *et al*,1983, Tojo *et al*,1988, Kitagawa,1992). It is possible that the microangiopathy and oedema are directly attributable to vitamin A deficiency, perhaps via the regulatory effect on growth factors (Gudas *et al*,1994). Fibroblast growth factors (FGFs) act directly on vascular cells to induce endothelial cell growth and angiogenesis (Nabel *et al*,1993) and may be regulated by retinoic acid.

Not only was the diet in this feedlot deficient in vitamin A it was also low in vitamin E and β-carotene, both recognized antioxidants (Rice and Kennedy 1988, Ross *et al* 1993). In various animal species, oedema and microangiopathy have been associated with free radical damage in vitamin E-selenium deficiency diseases. The vascular lesion in this study resembles the oxidative damage to blood vessels observed in "nutritional microangiopathy" or "mulberry heart disease" of pigs, a disease responsive to Vitamin E and "exudative diathesis" in chickens, a disease responsive to Vitamin E and selenium. There is a brief description in the scientific literature of observations made while attempting to experimentally induce white muscle disease (McMurray *et al*,1983). A vascular lesion occurred in two cattle which were low in Vitamin E and selenium and then fed a diet high in polyunsaturated fatty acids. Japanese researchers (Okuda *et al*,1983) observed a vascular lesion in vitamin A deficient cattle but did not measure vitamin E levels. Oedema is also seen in the human condition, kwashiorkor, which occurs with protein deficiency and an adequate caloric intake. It has been postulated that the various pathological changes observed may be related to oxidative damage as the sufferers are low in antioxidants. Oedema, particularly of the lower legs is one of the initial symptoms and although there is always hypoalbuminaemia, and a decrease in the total serum protein, this does not always correlate

well with the severity of the oedema. It has been observed that if diets formulated to "correct" this malnutrition contain polyunsaturated fatty acids (PUFAs) or iron the condition worsens and the death rate rises. Both PUFAs and iron exacerbate oxidative damage (Golden and Ramdath,1987).

It may be that the oedema is a result of a combined deficiency of vitamins A and E, and perhaps also β-carotene. A review of previous reports of oedema in vitamin A deficient cattle reveals that the diets in these cases were most likely also deficient in vitamin E and β-carotene.

Similarly an intolerance to heat stress is not recognised generally in vitamin A deficiency. Moore (1941) noted that "warm weather also seemed to be hard on deficient cows". Seawright (1966) noted that vitamin A deficient animals show an intolerance to heat in warm sunny weather, have elevated rectal temperatures and show respiratory distress and that vitamin A therapy alone returns the temperatures to normal. Vitamin A may have a direct role in thermoregulation or the physical consequenses of vitamin A deficiency may impair the body's ability to thermoregulate. Interestingly in one Japanese study (Okuda *et al,* 1983) deaths occurred in the winter. Perhaps the deficiency initiates a defect in thermoregulation and extremes of weather conditions cannot be tolerated.

Vitamin A and E requirements are high for rapidly growing animals (Jensen and Mackey 1971) and may be particularly high when the preference is for marbled meat with a high fat content. The requirement for vitamin A is greater in hot weather (Jensen and Mackey 1971) and it is conceivable from our observations that either vitamin A or E or both are required for effective thermoregulation. Perhaps some of the clinical signs such as the severe oedema and intolerance to heat stress could be prevented by increasing antioxidant levels. From this study the deficiency of vitamin A, probably in association with a deficiency of vitamin E resulted in unacceptable clinical consequences.

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References

Davis TE, Krook L and Warner RG (1970) Bone resorption in hypovitaminosis A. The Cornell Vet. 60:90-119.

Divers TJ, Blackmon DM, Martin CL and Worrell DE (1986) Blindness and convulsions associated with vitamin A deficiency in feedlot steers. J Am Vet Med Ass 189:1579-1582.

Golden MHN and Ramdath D. (1987) Free radicals in the pathogenesis of kwashiorkor. Proc Nutrit Soc 46:53-68.

Gudas LJ, Sporn MB and Roberts AB (1994) Cellular biology and biochemistry of the retinoids. In The Retinoids: Biology, Chemistry and Medicine. 2nd edition. Edited by MB Sporn, AB Roberts and DS Goodman. Raven Press, Ltd., New York 1994

Haq RU and Chytil F (1992) Effect of retinoids on nuclear retinoic acid receptors mRNA in adipose tissue of retinol-deficient rats. J Lipid Res 33:381-384.

Haq RU, Pfahl M and Chytil F (1991) Retinoic acid affects the expression of nuclear retinoic acid receptors in tissues of retinol-deficient rats. Proc. Natl. Acad. Sci. USA Biochem 88:8272-8276

Jensen R and Mackey DR (1971) Vitamin A deficiency "Diseases of Feedlot Cattle" 2nd Ed. pp 338-343

Kitagawa M (1992) The metabolism of vitamin A and lipid metabolism. Special Report- "Fattened Cattle and Vitamin A " in Clinical Veterinary Studies 10: No 11.

Madsen LL and Earle IP (1947) Some observations on beef cattle affected with generalized edema or anasarca due to vitamin A deficiency. J Nutrit 34:603-618.

McMurray CH, Rice DA, and Kennedy S. (1983) "Nutritional myopathy in cattle; from a clinical problem to experimental models for studying selenium, vitamin E and polyunsaturated fatty acids interactions" in Trace Elements in Animal Production and Veterinary Practice. Occasional Publication No. 7 - Brit Soc of Anim Prod 1983. pp 61-73.

Moore LA (1941) Some ocular changes and deficiency manifestations in mature cows fed a ration deficient in vitamin A. J Dairy Sci 24:893-902

Nabel EG, Yang ZY, Plautz G, Forough R, Zhan X, Haudenschild CC, Maciag T and Nabel GJ (1993) Recombinant fibroblastic growth factor-1 promotes intimal hyperplasia and angiogenesis in arteries *in vivo*. Nature 362:844-846

Okuda M, Senda H, Yamasita , Ida T, Miyamoto M, Miyamoto E and Yamaoka T (1983) Skeletal Muscular Edema Probably Caused by Hypovitaminosis A in Fattening cattle. J Jap Vet Med Ass 36: 528-533.

Rice DA and Kennedy S (1988) Vitamin E:function and effects of deficiency. Br Vet J 144:482-496

Ross AC and Ternus ME (1993) Vitamin A as a hormone: recent advances in understanding the actions of retinol, retinoic acid, and beta carotene. J Am Diet Ass 93:1285-1290

Safonova I, Reichert U, Shroot B, Ailhaud G and Grimaldi P (1994) Fatty acids and retinoids act synergistically on adipose cell differentiation. Biochem and Biophys Res Comm 204:498-502

Seawright D (1966) Avitaminosis in calves. Vet Rec 78:320

Tojo H, Knoshita S, Sato Y, Saruta K, Oota T and Takada T (1988) Vitamin A deficinecy in fattening Japanese black cattle. J Jap Vet Med Ass 2:108-113

Torii S, Matsui T and Yano H (1996) Development of intramuscular fat in Wagyu beef cattle depends on adipogenic or antiadipogenic substances present in serum. Anim Sci 63:73-76

Van der Lugt JJ and Prozesky L (1989) The pathology of blindness in new-born calves caused by hypovitaminosis A. Onderstepoort J Vet Res 56:99-109