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A new bovine disease, microangiopathy, related to low antioxidant levels in feedlot rations. Continued investigations into the pathogenesis

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ABSTRACT

This investigation aimed to determine the cause of an unexplained condition of leg swelling in feedlot cattle. Through a survey of Australian feedlots and investigations into spontaneous cases of this condition, possible causative factors were investigated. These factors included toxic agents, infectious agents, nutritional imbalance and climatic factors. Consistently low serum vitamin E levels were found in affected animals and the histopathogical findings supported the hypothesis of vitamin E deficiency. The condition occurred most commonly during periods of prolonged hot weather. A vitamin E response trial was conducted at a commercial feedlot when the condition arose. Additional supplementation of the diet alleviated the condition and apparently has prevented the condition recurring. It appears that vitamin E deficiency damages microcirculation in the lower leg and in turn this damage may impair the body's temperature regulation. From this study recommendations are made for the requirements of supplemental vitamin E for feedlot cattle under Australian conditions. Not only should these recommendations alleviate the condition of leg swelling but may also assist in the prevention of heat stress related deaths.

EXECUTIVE SUMMARY

Project objectives

. To investigate an unexplained condition of leg swelling of feedlot cattle which although not fatal can cause significant economic loss through the large number of cattle which may be affected at any one time and the disruption to normal management.

. To ascertain if the condition had any effect on thermoregulation.

. To make recommendations for treatment and prevention.

Significant results

Comprehensive investigations were carried out to determine the factors involved in this condition. This involved surveying the feedlot industry through a questionnaire and closely examining outbreaks of the condition in terms of clinical signs, disease pattern and possible causative factors.

Significant findings were:-

Association with low levels of vitamin E supplementation and hot weather
Consistently the condition occurred during prolonged hot weather in animals fed low levels of supplementary vitamin E, and which had low blood levels of vitamin E. Secondary dietary factors which may have exacerbated the condition were high levels of fat (tallow or whole cotton seed), high levels of carbohydrate, and lactic acidosis, rancidity, fungal contamination, nitrites, and ionophors. Blood levels of vitamin E were observed to drop as the number of days on feed increased, indicating that levels of supplementation were inadequate.

Response to dietary vitamin E supplementation
A response trial at one feedlot showed that dietary supplementation with vitamin E alleviated the condition and the adoption of a feeding regime including an increased supplementation of vitamin E has apparently prevented recurrence.

Description of the condition, clinically and histolopathologically
The condition was first observed as lameness, fluid swelling of the hock joint, progressing to generalised swelling down to the hoofs and sometimes up to the groin. The skin directly above the hoof and around the pastern was reddened, swollen and tight. Affected animals were inclined to have elevated temperatures. Briefly the histopathological changes included damage to cells lining small blood vessels with subsequent leakage of fluid, protein and occasionally blood into surrounding tissues. Subcutaneous blood vessels and those about the joints were most severely affected leading to inflammation, haemorrhage and oedematous swelling.

Association with deaths from hyperthermia
During investigations into leg-swelling, two feedlots in northern NSW and one in southern Queensland suffered many deaths from heat stress during days of prolonged hot, humid weather in February 1991. The two feedlots in northern NSW had both had recent outbreaks of "leg-swelling" investigated by the Regional Veterinary Laboratory Armidale and the feedlot in Queensland had a condition of redness and swelling in the legs in November and December 1990.
There is strong evidence from the findings of this project that these deaths from heat stress were related to low levels of supplementary vitamin E in the diet leading to damage of peripheral blood vessels in the limbs which, when functioning properly, have a vital function in temperature regulation.

Recommendations for vitamins E and A supplementation
The recommended levels of supplementary vitamin E have changed considerably over the last decade with the increased understanding of the role of vitamin E in enhancing the shelf-life of meat, and its role in alleviating stress related conditions and enhancing the immune system. Based on the findings of this project, recommendations are made for the levels of both vitamins E and A required for feedlot cattle in Australian climatic conditions.

Conclusions

Results from this study show that depletion of vitamin E in feedlot cattle is responsible for damage to small blood vessels in the limbs, particularly in the lower hind legs. It appears that this damage impairs the body's temperature regulation and hence results in an inability to cope with heat stress. The depletion of vitamin E is related to low dietary intake as well as other dietary factors which can affect vitamin E availability, uptake and utilisation.
The findings from this study have been disseminated through the rural press, breed societies and feedlot producers throughout Australia.

Recommendations for future actions

Further investigations are warranted into heat regulation, the role of the arteriovenous anastamoses and their susceptibility to damage, and more in-depth investigations into the physiological role of vitamin E, vitamin A its derivative, rhodopsin or similar compounds in thermoregulation.
Investigations are warranted into the consequences of low omega-3 fatty acid intake in ruminants. Current literature relates only to humans and other monogastric species.
There is a need to determine the most effective, cost efficient method of administering vitamin E and also to determine the rate of deterioration of vitamin E in premixes in Australian storage conditions.

ADMINISTRATIVE DETAILS REPORT

Financial statement

The Meat Research Corporation contributed $32,000 which was used for cost of laboratory testing, travel, data collection, report preparation. NSW Agriculture contributed professional and laboratory staff time and laboratory overheads estimated at approximately $40,000.

Intellectual property

None of the information in this project could be considered commercially sensitive or suitable for commercial exploitation. Throughout the project every effort was made to disseminate the findings and recommendations to the feedlot industry.

MAIN RESEARCH REPORT

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1 Background to the project

A summer associated condition of swelling of the hind legs has been reported in feedlot cattle in NSW and Queensland. A similar summer associated condition has been observed in North American feedlots - the cause has not been identified. Since the summer of 1990 and during following summers, samples from swollen legged cattle were submitted to the RVL Armidale for investigation.

Sporadic cases were reported but the greatest concern was when the condition occurred as a sudden outbreak with up to 30% of animals in the feedlot affected. Although fatalities were not reported, the condition caused interruption to normal management procedures and delays in finishing cattle which one feedlot veterinarian estimated to cost $100 per affected beast. The condition has only been observed in cattle that have been on the feedlot for greater than 60 days. Field observations suggested a relationship with weather patterns particularly to stormy hot humid weather of January.

Initially an ergot toxin was suspected. This was eventually discounted; no ergots could be demonstrated in the feed and the microscopic findings were not consistent with ergot poisoning, neither did the lesions appear to be due to an infectious agent and no organisms were isolated.

The microscopic changes seen in blood vessels were similar to those seen in pigs and chickens related to antioxidant deficiency. 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. At the RVL Armidale we have observed vascular damage in feedlot cattle with concomitant Vitamin A and E deficiency. Japanese researchers (Okuda *et al* 1983) have observed a vascular lesion in Vitamin A deficient cattle. In human medicine oxidative damage is implicated in diseases as diverse as arthritis and cardiovascular disease. The endothelial damage observed in these feedlot cattle with leg swelling may be similar in aetiology to the endothelial damage which precedes atherosclerotic plaques in humans.

As a consequence the Vitamin A and E status of a limited number of cattle with swollen lower legs was assessed. Levels were measured in both serum and liver in both normal and affected cattle. These preliminary investigations showed consistently that affected cattle had lower than normal serum and liver Vitamin E levels. Serum CPK and LDH values were elevated in severe cases. Glutathione peroxidase levels were normal. β-Carotene (also an antioxidant) was not measured but the lack of green feed in the diet suggests that these levels would be low year round. Some clinical response had been noted to injectable vitamin E.

Many factors will influence the dietary requirements of vitamin E. These include fatness, metabolism, ingested toxins, high carbohydrate rations, ingested and absorbed polyunsaturated fatty acids (PUFAs), and hot environmental conditions. Animals ingesting high levels of PUFAs have high Vitamin E requirements. PUFAs are normally hydrogenated in the rumen thus decreasing the potential harm they can cause through oxidative damage to cells. Any malfunction in the rumen which reduces hydrogenation (eg lactic acidosis) may allow the PUFAs to pass through the rumen for absorption in the small intestine. A sudden surge of PUFAs into the system can swamp the antioxidants including any available Vitamin E (Vitamin E is present in cell membranes to protect them against such damage).

Thus a similar set of conditions may be occurring as those in the experimental animals fed a diet high in polyunsaturated fatty acids. It is documented that feedlot cattle are more prone to lactic acidosis when there is a sudden drop in barometric pressure (Blood, Radostits and Henderson 1983). The dropping pressure stimulates excessive feed intake and lactic acidosis. In turn this could induce an oxidation overload. This may well explain the observation of explosive outbreaks in stormy weather.

To investigate the hypothesis that the leg swelling is related to low levels of vitamin E, the condition needed to be investigated further, including the measurement of vitamin E and PUFA levels in feedlot cattle. From studies of feedlot cattle unsupplemented with vitamins A and E, we have observed that these cattle appear to have defective thermoregulatory mechanisms and are susceptible to heat stress. It was not known whether the same situation applied when only Vitamin E was deficient. In view of the severity of

heat stress related deaths in Australia this aspect needed further investigation.
Treatment and prevention protocols needed to be developed.

2 Project objectives

- To investigate an unexplained summer associated condition of feedlot cattle (greater than 60 days on feed) manifest as lameness, swollen hind legs, and sometimes involving up to 30% of the feedlot.
- To determine the extent of the problem in Australian feedlots through a mailed survey via ALFA.
- To study the pathogenesis of this disease by investigating the possible role of infectious and nutritional causes including free radical formation and oxidative overload, polyunsaturated fatty acid absorption, lactic acidosis as well as feed content, quality and possibly toxicity.
- To determine if this condition is associated with poor thermoregulation.
- To suggest treatment and prevention regimes and monitor responses.
- To fully describe the histopathological changes.
- To write a literature review with specific reference to the aetiology of the condition and the role of antioxidants.

3 Detailed methodology

PUFA analysis - conducted at CSIRO Food Processing Division, Meat Research Laboratory, Cannon Hill, Qld. by Dr. Ron Tume
Extaction of lipid: Lipid is extracted from sera by the Folch wash method using chloroform:methanol, 2:1, v/v. (Folch 1957).
Preparation of fatty acid methyl esters: The lipid containing phase is evaporated to dryness then esterified using a modified procedure of Bannon *et al*,(1982) using 0.25 M sodium methoxide and diethyl ether 1:1.
Fatty acid analysis by Gas Chromatography: The petroleum ether extract is reduced to a suitable volume for fatty acid analysis on a Shmadzu GC 17A Gas Chromatograph fitted with a Chrompack WCOT Fused Silica capillary column.

Vitamin E, vitamin A analyses - conducted at NSW Agriculture, Elizabeth Macarthur Agricultural Institute
For tissues and feed, the material is saponified in an alkaline aqueous medium containing ethanol or methanol. This method converts all vitamin E esters to dl-alpha-tocopherol which is then extracted by petroleum ether. DL-alpha-tocopherol is then quantitated by reverse-phase HPLC equipped with fluorescent and UV detector. For plasma/serum the saponification in alkaline aqueous medium is omitted.

Other biochemical analyses - performed at RVL Armidale using a Cobas Mira auto analyser (Roche)

Histopathology, and bacteriology were performed using standard methods at the RVL Armidale.

Haematology performed at RVL Armidale with cell counts performed on an automated cell counter (Cell-Dyn 400 - Sequoia-Turner) with differential counts done on examination of smears.

The serological test for Chlamydia antibody was performed at RVL Armidale using a standard complement fixation test.

4 Results and discussions

4.1 Results from the Questionnaire (Refer to Appendix 1)

From the replies received from 15 feedlots, 9/14 feedlots have seen the condition. Assuming that at least 75 feedlots received the questionnaire, a minimum of 9/75 (i.e. 12%) have experienced this problem.
In an affected feedlot the incidence may be as low as <2% to 30-40%.

The condition has been seen in both sexes including entire and castrated males.

The condition can occur at any time of the year but predominantly in the summer months.

The condition is sporadic and unpredictable.

It occurs usually between 60 and 160 days on feed, but the earliest mentioned was 30 days on feed, and the latest 300 days on feed.

Two comments from veterinarians implicate rancid tallow.

Both feedlots feeding tallow had seen the problem as did both feedlots feeding whole cotton-seed (high oil content).

There is some indication that the condition is related to low vitamin E levels in the diet.

From comments from veterinarians, a number of conditions may be covered by the terms "swollen hock" "swollen legs". These include:-

i. The sudden appearance of reddening and subcutaneous swelling of hind legs, usually from hock to coronary band and swelling of hock joint. Cause unknown (The subject of this investigation).

ii. Vitamin A deficiency which progresses to a generalised swelling of all 4 legs, brisket and submandibular region.

iii. Weight related or traumatic arthritis of hock joint.

iv. Nutritional or toxic myopathy.

For this reason it is possible that the responses received from feedlots may have included conditions other than the one we wished to investigate. With vitamin A deficiency and myopathy, other signs would be manifest as well as the leg swelling.

Only 10 of the 15 feedlots which replied to the questionnaire revealed the level of vitamins A and E in the ration or the levels of vitamins A and E given at induction.
Only 11 of the feedlots revealed their feed intake.

Because many feedlots treat the details of their rations as confidential there have been great limitations to the amount of information that could be gathered in relation to this problem.

4.2 Description of the condition

The condition is first observed as lameness, fluid swelling of the hock joint, progressing to generalised swelling down to the coronary band and sometimes up to the groin. The skin above the coronary band and around the pastern is reddened, swollen and tight. Affected animals were generally greater than 60 days on feed (dof) with cattle less than 60 dof apparently not at risk.

Sporadic cases of this condition of hindleg swelling have been reported but the greatest concern is when the condition occurs as a sudden outbreak with up to 30% of animals in the feedlot affected. For example one feedlot identified as feedlot 16 in this study, experienced large outbreaks. Feedlot 16 with a total population of 6500 had an estimated 30% of cattle affected in January 1990 and an estimated 20% of cattle affected in January 1991.

FIGURE 1: Feedlot steer (feedlot 16) with marked thickening of the subcutaneous tissue overlying the lower hind legs from the hock to the hoof.

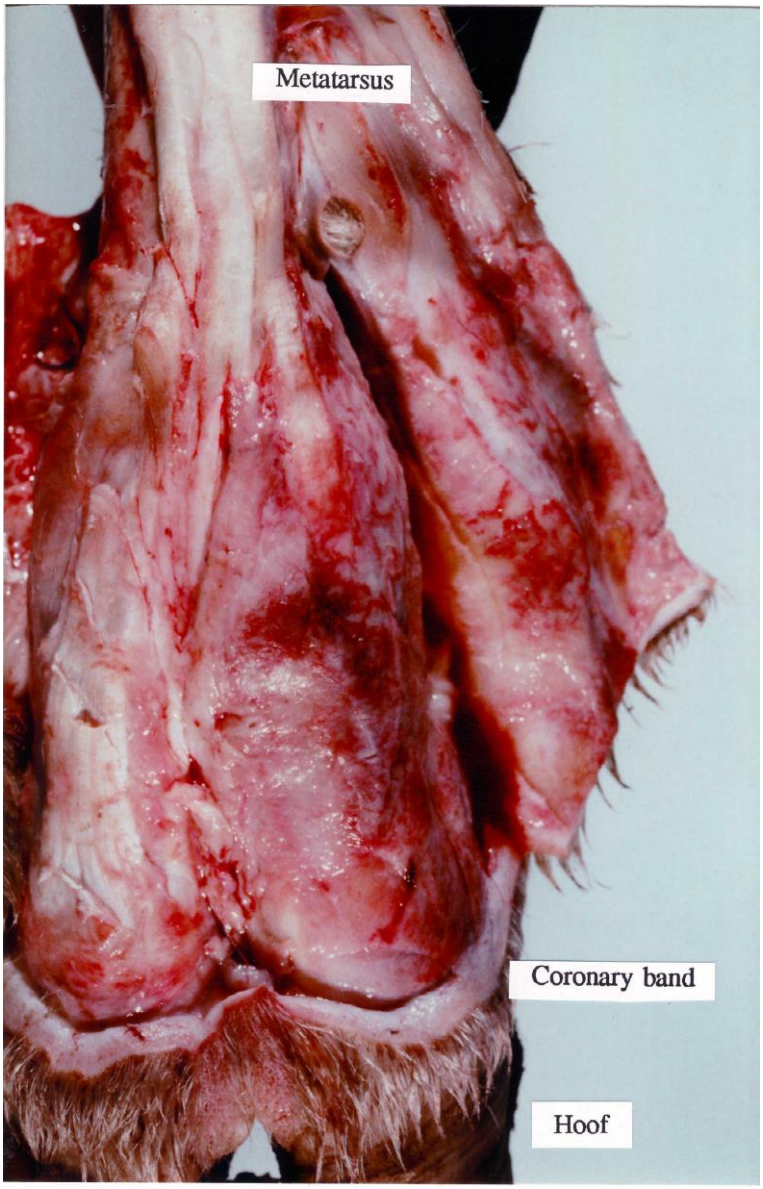

FIGURE 2: Lower hindleg of affected steer (feedlot 16). Thickened oedematous subcutaneous tissue, with fluid exudation, areas of haemorrhage, vascular congestion and accentuation of vasculature.

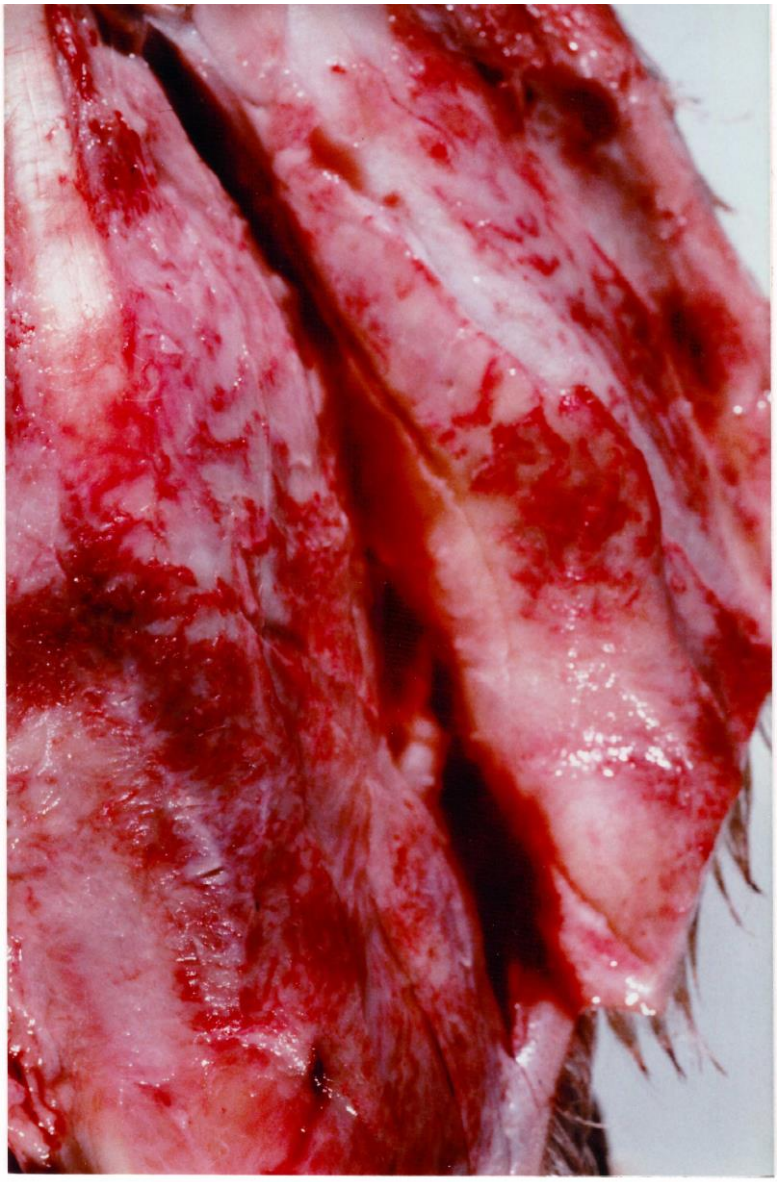

FIGURE 3: Higher magnification of leg in FIGURE 2.

4.3 Histopathological description of the vascular lesion

Microscopically there is damage to cells lining small blood vessels with subsequent leakage of fluid, protein and occasionally blood into surrounding tissues. Small subcutaneous vessels appear accentuated and proliferated. In many, the lumen appears occluded. Superficial blood vessels and those about the joints are most severely affected leading to inflammation of vessel walls and surrounding tissues, haemorrhage and oedematous swelling. Hyaline degeneration was observed in some vessel walls.

The vascular lesions resemble lesions observed in a disease of pigs: "Nutritional microangiopathy" or "Mulberry Heart Disease" although the anatomical location of the damaged blood vessels is different from that observed in pigs. Mulberry Heart Disease is metabolically induced causing damage to blood vessels in various organs and is responsive to Vitamin E. The review of the literature on vascular leakage and oedema related to oxidative damage describes a number of human conditions and a condition in chickens known as exudative diathesis, a condition alsoresponsive to vitamin E. There is a brief description in the scientific literature of observations made while attempting to experimentally induce white muscle disease in cattle. 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. The anatomical location of these lesions was not described.

At the RVL Armidale we have observed vascular damage in cattle with concomitant Vitamin A & E deficiency (Ref. Appendix 2) and Japanese researchers have observed a vascular lesion in Vitamin A deficient cattle.

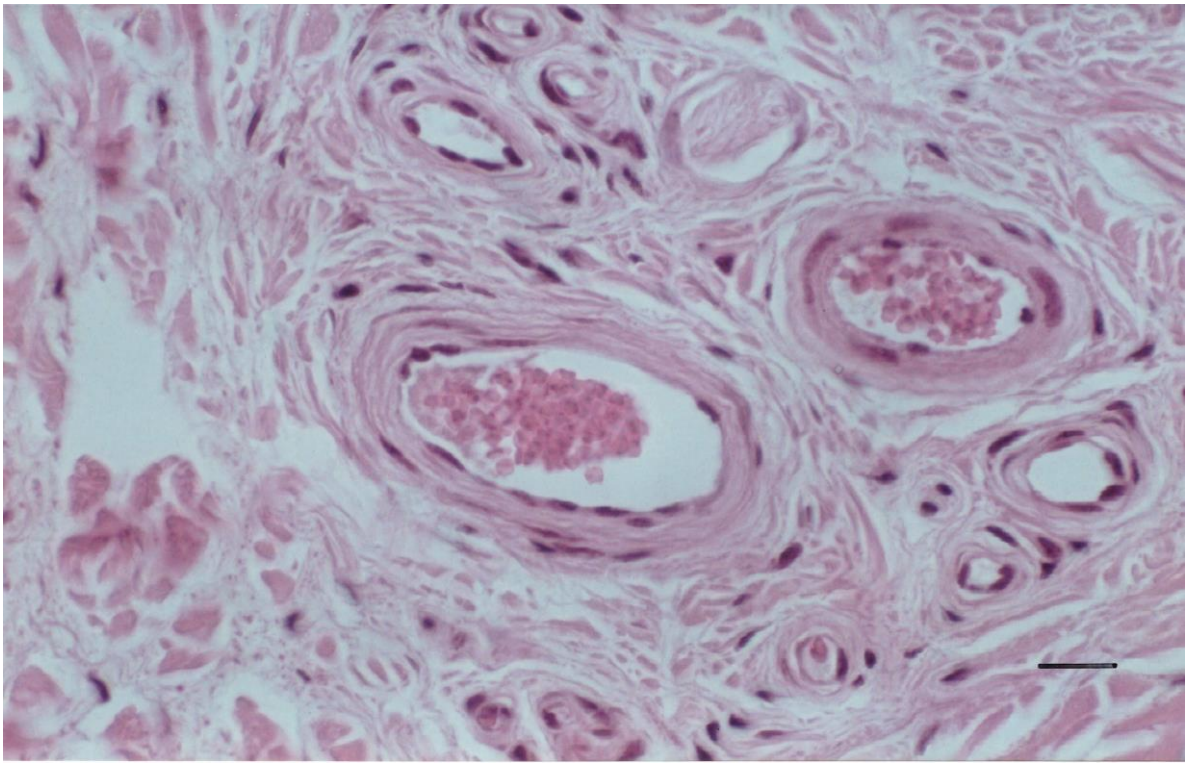

FIGURE 4: Normal blood vessels in subcutaneous tissue of lower hind leg of unaffected bovine. 1cm bar = 3.8μm

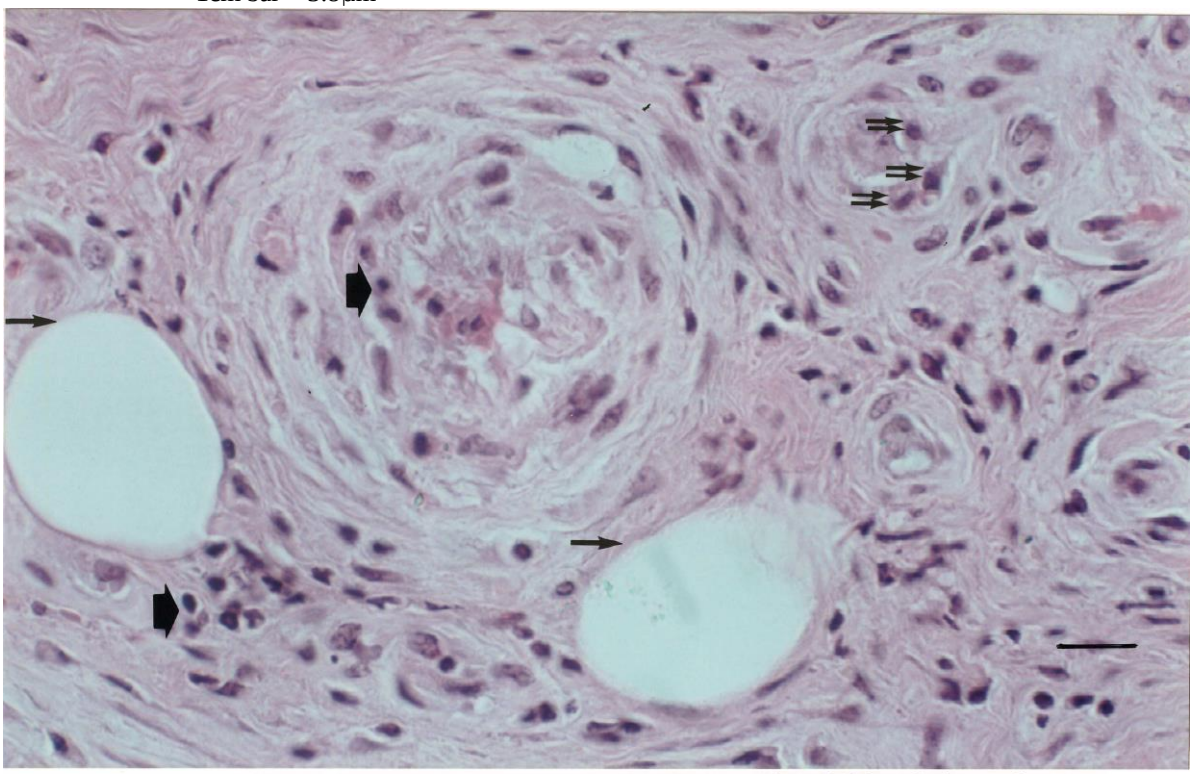

FIGURE 5 : Subcutaneous tissue from lower leg of affected steer. Distended lymphatics, oedematous blood vessel walls with swelling of endothelial cells, occlusion of lumen and inflammatory cells within and outside vessel walls. 1cm bar = 3.8μm

→ Dilated lymphatics
⇉ Swollen endothelial cells
➧ Inflammatory cells

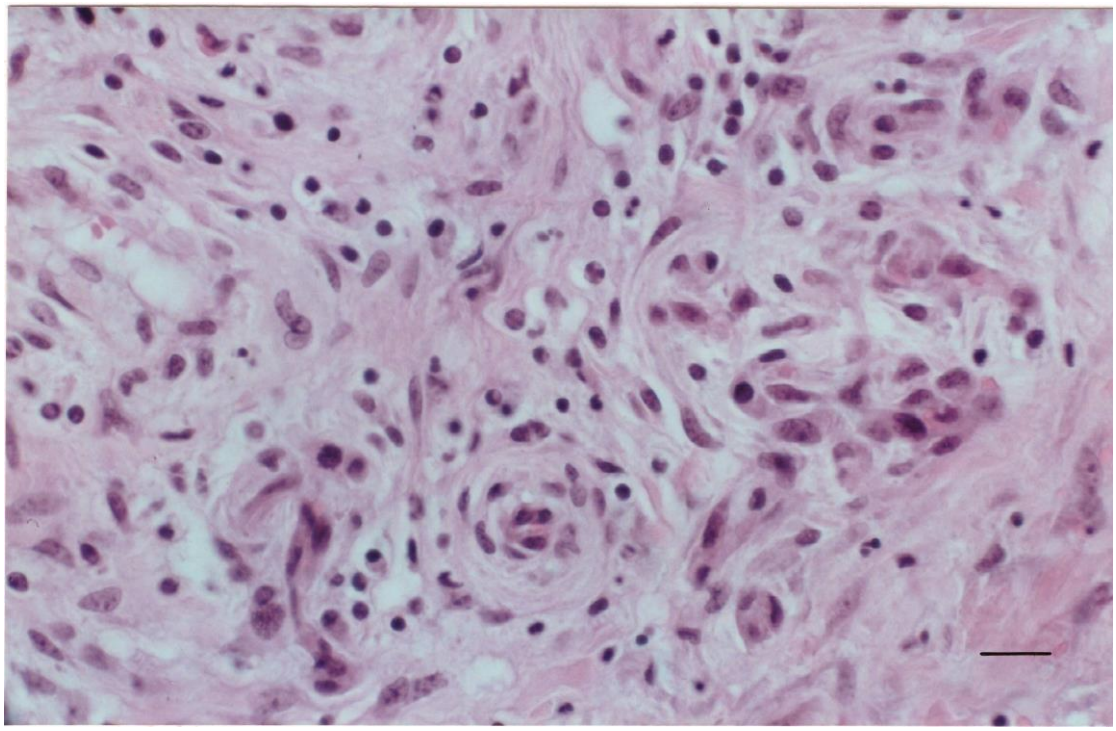

FIGURE 6: Abnormal blood vessels from affected steer. Marked inflammatory response. 1cm bar = 3.8μm

4.4 Investigations into factors which may be involved in vasculitis and vascular degeneration in the limbs

The following factors were considered in the investigations into this condition:-
Toxic agents
Infectious agents
Immune mediated reactions
Nutritional imbalance

4.4.1 Toxic agents

Ergot toxicity. Grain samples were examined from 3 affected feedlots - no ergots were found. No areas of gangrene were noticed in the extremities of any cattle. The histopathological findings were not consistent with ergot poisoning i.e. there was no infarction.

Fungal toxicity. These grain samples were also examined for fungi. At feedlot 16, grain was contaminated with fungi.
At Feedlot 1 sorghum grain was examined and found to be contaminated with Aspergillus fungus but was not toxic when fed to mice.

Nitrate/nitrite toxicity. One silage sample from feedlot 8 was positive for nitrate/nitrite. A second sample was negative.

None of these agents were considered to be the primary cause of the condition but both fungi and nitrates can increase the utilisation of antioxidants in feed.

4.4.2 Infectious agents

Various infectious organisms were included in the differential diagnosis of the condition.

These included:-
Non-specific bacterial infections
Specific bacterial infection - *Haemophilus somnus*
Chlamydial infection
Mycoplasma infection
Virus infection e.g. arboviruses - Bluetongue, Bovine ephemeral fever.

Tissues and fluids were collected from affected cattle from feedlots 1, 5 and 16.
No significant bacteria were isolated, mycoplasma was not isolated and serum samples were negative for chlamydia antibodies.

Arboviruses e.g Bluetongue virus and bovine ephemeral fever. No arbovirus activity has been detected in the region of 3 of the closely monitored affected feedlots: feedlot 1, 16 and 5 this decade based on sentinel herd testing data (personal communication from Peter Kirkland, NSW Agriculture, Elizabeth Macarthur Agricultural Institute).

Tissues from affected limbs were collected for histopathological examination. No infectious organisms were detected using special staining techniques.

Routine haematological parameters measured on blood samples. The majority of samples tested showed no abnormality but in some cases there was an elevated white cell count with neutrophilia.

From the samples examined no significant infectious agents were identified. Haematological examination of the blood samples did not suggest an infectious cause.

4.4.3 Immune mediated reactions

Immune reactions are less important primary causes of vasculitis in domestic animals than they are in humans. It was felt that this type of reaction was unlikely to be involved because this condition of leg swelling has been seen to affect large numbers of cattle at one time.
Immune reactions can be exacerbated by an imbalance of omega 6 and omega 3 fatty acids in the diet of monogastric animals. The possibility exists that the imbalance demonstated in this study may be a factor in the pathogenesis of the condition.

4.4.4 Nutritional imbalance

Vitamin A deficiency

A syndrome of concomittent vitamin A and E deficiency has been observed in feedlots feeding cattle long-term for the Japanese market. One of the signs observed was swelling of the legs which could become so severe that oedema was present in both front and back legs and extended to the brisket and neck (refer to Appendix 2). In the syndrome of hindleg swelling which is the subject of this current investigation, vitamin A deficiency was ruled out. Adequate levels of vitamin A were included in the rations and blood levels and tissue levels were normal for the majority of samples tested.

Antioxidant deficiencies

Selenium and glutathione peroxidase. Glutathione peroxidase was measured in blood samples collected from 4 feedlots (1,5,16,17). The levels were all good (greater than 100 U/g haemoglobin) reflecting the good levels of selenium in the soil of the major grain growing areas.

β-carotene. Serum levels were measured in samples from 3 feedlots (1,5 and 17). These were low, reflecting the lack of green feed in the rations. In general β-carotene levels were below 1 μmol/l whereas normal values for animals on green feed would be approximately 4-15 μmol/l. Low levels of this antioxidant would increase the demand on other antioxidants. With the current market requirement for white fat there is little scope for increasing the levels of β-carotene as this would make the fat yellow. This market force may of course change with time especially with the increasing scientific understanding of the role of β-carotene as an antioxidant.

Vitamin E. Low serum vitamin E levels were found in the majority of affected cattle. (Refer to section 4.5.6)

Feeds and additives which may affect antioxidant levels in the animal

Fat content in feed
From the questionnaire results of the 15 feedlots, the 2 feeding tallow and the 2 feeding whole cotton-seed (high oil content) had seen the condition.
Feedlot 17 (not in the questionnaire) fed 5% whole cotton seed and had the condition.
Cottonseed contains approximately 25% polyunsaturated fat. It should never be fed to young preruminant animals and should not be fed at greater than 15% of the diet (pers. comm. Gary Holcomb - nutritionist, Toowoomba 1992)
Animals ingesting high levels of polyunsaturated fatty acids (PUFAs) have high Vitamin E requirements.

Rancidity
From the results of the questionnaire two feedlot veterinarians associated the condition with the feeding of rancid tallow. The addition of an antioxidant to the tallow appears to have alleviated the problem. Rancidity depletes antioxidants in feed as well as producing toxic compounds which are absorbed through the gut. Pyrogens may be formed from the oxidation of oil.

High levels of carbohydrate and lactic acidosis
Lactic acidosis causes the ruminal microflora to malfunction thus reducing the biochemical activity. Hence one function, the hydrogenation of PUFAs, would be expected to be reduced. PUFAs that are absorbed into the body are a potent source of free radicals and hence oxidative damage to cells. PUFAs are normally

hydrogenated in the rumen thus decreasing the potential harm they can cause. If PUFAs pass through the rumen for absorption in the small intestine then a sudden surge of PUFAs into the system can swamp any available Vitamin E (Vitamin E is present in cell membranes to protect them against such damage).

For feedlot 5 samples of rumen contents were collected at the abattoir from cattle that had shown signs of leg swelling. In addition the rumen lining was examined for signs of chronic lactic acidosis.
The mean pH for ten rumen fluids was 7.15 (SD ±0.18).
Both the rumen pH measurements and the examination of the rumen did not indicate that the animals currently had or had previously had lactic acidosis. Although in this study, lactic acidosis has not been demonstrated as a causative factor in leg swelling, it could exacerbate the condition.

Preliminary results in this current project demonstrate that serum samples from cattle with clinical lactic acidosis have elevated PUFA levels. Blood samples were collected from cattle with leg swelling and from cattle at a similar stage in the feedlot, 3 weeks later with clinical acidosis. These samples were fortuitously collected from clinically ill animals and were not part of a controlled experiment. Nevertheless there is a significant difference in the 18:2 (an omega 6 fatty acid) and the total of 18:2 and 18:3 (an omega 3 fatty acid) PUFAs in the serum with the acidotic cattle having the higher level (See Table 1).

Table 1: Serum linoleic (18:2) and linolenic (18:3) levels as a % of total fat in cattle without acidosis (but with leg swelling) and cattle with clinical acidosis.

Animal Id	History	Date	18:2	18:3	18:2 + 18:3
94/409 1	Feedlot 5 >250 dof Leg swelling	2/2/94	29.3	1.8	31.1
4	"	"	37.4	1.2	38.6
7	"	"	41.4	1.2	42.6
8	"	"	28.5	1.1	29.6
Mean (±SD)			34.2 (±6.3)	1.3 (±0.3)	35.5 (±6.2)
94/707 1	Feedlot 5 >200 dof Lactic acidosis	21/2/94	44.4	1.0	45.4
2	"	"	46.4	1.4	47.8
4	"	"	48.2	1.7	49.9
6	"	"	48.9	1.1	50
			47.0 (±2.0)	1.3 (±0.3)	48.3 (±2.2)

Statistical analysis showed that for the cattle with lactic acidosis versus the cattle with leg swelling there was a significant difference ($p<0.05$) in 18:2 levels and (18:2+18:3) levels.

Serum PUFAs

Serum samples were collected from various feedlot cattle including cattle with leg swelling, cattle with clinical acidosis and normal cattle at slaughter. Consistently they all had very low levels of linolenic acid (See Table 2) compared with pasture fed or hay fed cattle (See Table 3). Grains have high linoleic: linolenic ratios. The cattle at slaughter had the lowest levels of linolenic acid which most likely reflects the high level of grain in the finisher ration.

Ionophors - such as Monensin. These have been shown to increase vitamin E requirements. Ionophors are commonly included in feedlot diets. From the 10 feedlots that replied to the question :- "Are other feed additives included eg Monensin and Lasalocid?" all 10 replied "yes".

Table 2: Serum fatty acid levels as a proportion of total fatty acids (%,w/w) in feedlot cattle.

Animal Id	History	Date	16:0	18:0	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	18:2/ 18:3
94/409 1	Feedlot 5 >250dof Leg swelling	2/2/94	18.7	14.5	20.5	29.3	1.8	16.3
94/409 4	Leg swelling	"	16.8	14.6	16.4	37.4	1.2	31.2
94/409 7	Leg swelling	"	15.5	16.8	12.4	41.4	1.2	34.5
94/409 8	Leg swelling	"	20.2	13.2	23.9	28.5	1.1	25.9
94/793 1	Feedlot 1 Leg swelling	25/2/94	16.5	15.7	16.4	35.0	3.1	11.3
94/793 3	Leg swelling	"	18.4	15.4	21.6	30.5	2.0	15.3
94/793 4	Leg swelling	"	19.5	13.0	26.7	25.6	2.2	11.6
94/793 5	Leg swelling	"	15.8	13.9	19.3	35.4	2.8	12.6
94/707 1	Feedlot 5 >200 dof Lactic acidosis	21/2/94	13.8	14.3	12.8	44.4	1.0	44.4
94/707 2	Lactic acidosis	"	14.2	14.6	12.8	46.4	1.4	33.1
94/707 4	Lactic acidosis	"	14.4	12.3	13.6	48.2	1.7	28.4
94/707 6	Lactic acidosis	"	13.1	11.9	12.4	48.9	1.1	44.5
92/3464 4	Feedlot 5 300 dof	8/10/92	14.7	23.7	16.8	31.2	0.7	44.6
92/3464 2	"	"	11.3	14.7	10.9	51.5	0.3	171.7
92/3464 1	"	"	11.6	16.5	11.5	48.1	0.3	160.3
92/3464 5	"	"	12.2	16.2	10.6	47.4	0.5	94.8
92/3464 3	"	"	12.7	17.7	14.6	37.3	0.5	74.6
92/3464 23	"	"	11.9	18.5	8.2	48.9	0.3	163.0
92/3464 25	"	"	12.2	16.4	12.3	45.6	0.2	228.0
92/3464 21	"	"	11.1	15.6	9.5	49.9	0.2	249.5
92/3469 24	"	"	12.2	17.6	9.4	48.6	0.3	162.0
92/3469 22	"	"	10.9	14.3	9.9	51.5	0.3	171.0

Table 3: Means of serum fatty acid as a proportion of total fatty acids (%, w/w) in Spring calving Friesian heifers at four times of the year. (Wichtel *et al,* 1996)

	18:2	18:3	18:2/18:3
Pasture			
June	19.2	12.7	1.5
July	21.1	15.7	1.3
August	15.6	13.5	1.2
October	21.1	23.3	0.9
Hay			
June	19.3	12.8	1.5
July	17.5	9.9	1.8
August	16.2	14.4	1.1
October	20.9	22.3	0.9

4.5 Investigations into the role of vitamin E

4.5.1 Vitamin E levels in stored feed

Vitamin E is quickly depleted in stored feed with the deteriotation accelerated in hot weather (Lynch 1991b, Coelho 1991a,1991d).

At feedlot 5 in March 1991, 2 samples of finisher feed without the inclusion of any vitamin supplement, had vitamin E levels of 13.6 um/kg (5.8 IU/kg) and 15.8 umol/kg (6.8 IU/kg). Assuming that the cattle eat approximately 10kg per day this unsupplemented feed would supply only 58-68 IU vitamin E per head per day. This is much lower than the current understanding of daily requirements for cattle. Refer to Section 7.1 for daily requirements of feedlot cattle ranging from 150 - 500 IU /animal/day.

4.5.2 Vitamin E supplementation

The levels of vitamin E and vitamin A supplementation were assessed for feedlots with and without the syndrome of leg-swelling. This was done by recording the levels specified in the ration or by measuring the levels in the vitamin/mineral premix or the levels in the final ration. (Refer to tables 4 and 5)
Table 4 includes the feedlots that had experienced leg-swelling and were closely monitored. This table also includes the records of deaths from heat stress.

The condition of swollen legs occurred when supplementary vitamin E was low (13-72 IU/animal/day) and the ratio of A:E was in most cases above 1000:1.
Note that for feedlot 5 there is a large discrepancy between the level of vitamin E specified in the ration and the actual amount in the premix. (160 IU versus 72 IU)

Vitamin A and E levels were also recorded for feedlots that had not had leg-swelling. See Table 5. This data was compiled from replies to the questionnaire in this project (Refer to Appendix 1). The levels of supplementary vitamin E ranged from 90 - 911 IU/ animal/day. The ratios of vitamin A:E were all lower than 1000:1. (cf Table 4: vitamin E supplement 13 -72 IU/animal/day and the majority of ratios of A:E >1000:1)

The recommended levels of supplementary vitamin E have changed considerably over the last decade with the increased understanding of the role of vitamin E in enhancing the shelf-life of meat, and its role in alleviating stress related conditions and enhancing the immune system. In 1982 the recommended level was 20-50 IU of supplemental vitamin E per head daily during the finishing period (Adams, 1982). In 1994 the recommended levels for enhancing meat preservation were 500 IU per head daily for the last 100 days of feeding (Smith, 1994). Others have recommended even higher levels of 1200-1300 IU per head daily for the last 38 -67 days of feeding (Arnold *et al*, 1993 and Mitsumoto *et al* 1991).

Table 4: Vitamin A and E levels in feed in relation to the occurrence of hind leg swelling and deaths from heat stress.

Feed-lot Id.	Date vitamin level recorded	Vit.A (IU/anim/day)	Vit.E (IU/anim/day)	Ratio A:E	Swelling of hindlegs	Heat stress deaths
1	- Jan 94 Jan 94	NA 45000* 22440***	0* 0* 69***	NA 325:1	Yes (Nov 90) Yes (Jan, Feb 94)	Yes (Feb 91)
5	Feb 92 Feb 94	0* 45000* 43348**	0* 160* 72**	 313:1 602:1	Yes (April 92) Yes (Feb 94)	No
8	Apr 91 Apr 91	34600* 48880**	15* 19.5**	2307:1 2507.1	Yes (Dec 90)	Yes (Feb 91)
16	Mar 91 Mar 91	100,000** 72,560***	29** 57***	3448:1 1275:1	Yes (Feb 90, Jan 91)	Yes (Feb 91)
17	Dec 93	45,000*	32.5*	1385:1	Yes	No
18	Feb 92	51,894**	13.2**	3931:1	Yes (Feb 92)	No

NA - not available
* Levels specified in ration
** Levels measured in premix and converted to levels in final ration
*** Levels measured in finisher ration

Table 5: Vitamin A and E levels in feedlot rations where the syndrome of hindleg swelling is not observed (compiled with data from replies to the questionnaire).

Feedlot Id.	Date vitamin level recorded	Vit A (IU/anim/day)	Vit E (IU/anim/day)	Ratio A:E
3	1994	30,000	90	333:1
5	1995	45,000	360	125:1
6	1994	47,500	143	332:1
8	1994	42,000	911	46:1
9	1994	28,800	200	14:1
11	1994	66,500	133	500:1

4.5.3 Seasonal variation in serum vitamin E levels

Refer to Table 9. At feedlot 16 serum vitamin E levels were significantly higher in September than in February. This may reflect the increased demand for vitamin E during warmer weather. It may also reflect different levels of natural vitamin E in feeds at different times of the year, for example storage in hot weather may rapidly deplete vitamin E. Naftalin (1954) demonstrated that low environmental temperatures reduced the level of oxidative damage in livers of vitamin E / selenium deficient rats. To determine accurately the effect of seasonal changes a controlled trial would need to be done where the ration remained constant throughout the year.

4.5.4 Serum levels of vitamin E in relation to days on feed

Serum vitamin E levels were shown to decline in relation to the number of days on feed at Feedlot 5 when feeding vitamin E supplement at a rate of 100 IU per head per day. This reflects increasing requirements of vitamin E either because of increased body weight and fat or because of changes in the ration such as an increased level of grain (Table 6).

Table 6: Serum levels of vitamin E in relation to days on feed at feedlot 5

Date	Days on feed	Number of samples tested	Serum vitamin E (umol/l)
November 1991	150	9	14.4 (±3.8)
"	180	10	13.7 (±2.5)
"	270	3	11.4 (±2.3)
January 1992	120	25 (8 pools)	12.2 (±2.1)
	210	9 (3 pools)	8.4 (±1.9)
	240	10 (3 pools)	9.7 (±1.3)
	330	3 (1 pool)	9.4 (0)

Results expressed as Mean (± SD)

The samples collected in January 1992 were pooled as shown in the table.

4.5.5 Effect of removing vitamin E supplement

In February 1992 at feedlot 5, the vitamin supplement was taken out of the ration. The levels of feeding had been vitamin E at 100 IU/head/day and vitamin A at 45,000 IU/head/day.

Samples of blood were collected just prior to vitamin supplement being left out and again one month later.

By April 1st 5 steers 150 days on feed had swollen hocks, elevated rectal temperatures and swollen hocks. The weather conditions were hot. Refer to Table 7.

Vitamin A was also taken out of the ration but because vitamin A is stored in the liver in large quantities it would take many months for it to be critically depleted (refer to Appendix 2). Serum vitamin A levels remained in the normal range (Refer to Appendix 4).

Table 7: The effect of removing vitamin supplement from the ration on serum vitamin levels and clinical signs.

Date	Clinical signs	Serum vitamin E (μmol/l)	Serum vitamin A (μmol/l)
February 92 (date supplement removed) (10 samples pooled)	Nil	12.1	1.8
March 92 (10 samples pooled)	Nil	7.8	1.5
April 92 (5 samples pooled)	Leg swelling	6.8	1.1

4.5.6 Serum and liver vitamin E levels in cattle with leg-swelling.

Serum vitamin E and A levels were monitored in cattle with leg-swelling from 4 affected feedlots. In some of these animals liver vitamin levels were also monitored.
Serum vitamin E levels generally were low to deficient and liver vitamin E levels were low to deficient in about half of those tested. Refer to Appendix 4 for values considered normal. Serum vitamin A was low in a small number of cases (Refer to Table 8, vitamin A levels marked with *). These same animals also had extremely deficient serum vitamin E. Vitamin A is carried in the blood stream from the liver to the rest of the body as a complex of retinol attached to the protein, retinol binding protein (RBP) and thyroxide binding prealbumin (Frye *et al,* 1991). When serum vitamin E levels are low this protein may be susceptible to oxidative damage thus depleting serum vitamin A. All liver vitamin A levels were normal.

Table 8: Serum vitamin E levels in swollen leg cattle

Feedlot Id.	Date	Serum vit. E (μmol/l)	Serum vit. A (μmol/l)	Liver vit. E (μmol/kg)	Liver vit. A (μmol/kg)
1	1/92	7.10	1.75	10.9	146.6
"	"	2.61	1.43	10.9	1254.0
"	1/94	10.91	1.79		
"	"	8.00	1.31		
"	"	4.73	1.65		
"	"	3.27	1.21		
"	"	7.45	1.20		
"	"	7.64	1.64		
"	"	10.18	1.96		
"	"	8.73	1.88		
5	4/92	6.76 (5 pooled)	1.10 (5 pooled)		
"	3/93	1.5 (3 pooled)	0.19 (3 pooled)*	2.29	154.55
"	2/94	4.7	0.35 + *		
"	"	6.7	1.61		
"	"	5.0	1.79		
"	"	4.7	0.74		
"	"	7.1	2.00		
"	"	5.6	1.12		
"	"	3.9	1.40		
"	"	2.4	0.84*		
"	2/94			14.4	246
"	"			8.6	311
"	2/94	3.84	1.33	6.51	250
"	"	2.02	0.96	2.73	271
16	3/91	1.4	0.4*	15.6	1020
"	11/91	4.1	0.5*		
"	"	3.56	0.95		
"	"	4.66	1.24		
"	"	9.5	0.92	18.7	336
"	"	4.7	1.00	1.8	282
"	"	7.7	0.79	12.6	488
"	"	6.4	0.95	10.7	293
"	"		0.66*	15.6	371
"	12/91	5.81	0.57*		
"					
17	1/94	11.99	2.64		
"	"	5.45	1.82		

+ This animal also had oedema around shoulders.

* These animals had marginal to low serum vitamin A.

The vitamin A and E levels in serum and liver of affected cattle were compared with unaffected cattle. From feedlot 16 affected cattle and unaffected cattle of a similar age were sampled in February 1991 and compared with random samples collected in September 1991 (Table 9).

Table 9: Vitamin A and E levels in serum and liver of cattle with leg-swelling compared with unaffected cattle, feedlot 16.

Clinical signs	Serum vitamin E (μmol/l)	Serum vitamin A (μmol/l)	Liver vitamin E (μmol/kg)	Liver vitamin A (μmol/kg)
Swollen legs- Feb 91	9.5	0.82	18.7	336
"	4.7	1.00	1.8	282
"	7.7	0.79	12.6	488
"	6.4	0.95	10.7	293
"	6.9	0.66	15.6	371
	Mean = 7.0^{a} (±1.8)	Mean = 0.8^{d} (±0.1)	Mean = 11.9^{f} (±6.4)	Mean = 354^{h} (±83)
Randomly selected- Feb 91	9.9	1.29	12.4	534
"	9.7	1.85	5.9	206
"	8.4	1.03	6.5	129
"	7.9	1.64	11.9	329
"	12.4	1.35	9.7	338
	Mean = 9.7^{b} (±1.8)	Mean = 1.4^{e} (±0.3)	Mean = 9.3^{g} (±3.0)	Mean = 307^{h} (±154)
Randomly selected 9/91	17.6	1.1	16.9	387.9
"	14.8	0.9	28.5	931.6
"	10.0	1.3	14.3	262.9
"	13.2	1.0	6.5	444.1
"	13.5	0.6	14.3	362.4
	Mean = 13.8^{c} (±2.8)	Mean = 1.0^{d} (±0.3)	Mean = 16.1^{fg} (±8.0)	Mean = 478^{h} (±262)

Mean levels in the same column with different superscript values are statistically different at $p \leq 0.05$.

4.5.7 Response of "swollen leg" cattle to supplementary vitamin E

Response to oral vitamin E

In January 1994, a total of 80-100 animals at feedlot 5 were found with leg swelling over approximately 10 days. Approximately 8-10 cases were identified each day. Most of those affected were between 270 and 300 dof. Clinical signs included swelling of hind legs particularly near hock with subcutaneous fluid evident. Some animals had a short proppy gait resembling founder.

This feedlot had commenced incorporating Monensin in the feed in November 1993 at a rate of Monensin 10ppm in Starter ration, 20ppm in Grower and Finisher ration. The vitamin supplementation was specified at 45,000 IU vitamin A per head per day and 160 IU vitamin E per head per day. Some animals were treated with Vitajec with poor results.

Actual vitamin E levels in the premix were much lower than in the specifications : 72 IU vitamin E /head/day rather than 160 IU/head/day specified (Refer to Table 4).

A response trial was conducted at Feedlot 5 in February 1994 as cases of leg-swelling continued to occur following the initial reports in late January.

Materials and methods

Animals

Control Group - A group of 25 affected "swollen legs" animals - no treatment.

Treatment Group - A group of 25 affected "swollen legs" animals -500 IU vitamin E/head/day.

Vitamin E

Vitamin E was kindly supplied by Fred Schwenke of Colborn-Dawes Australia (Pty) Limited as Rovimix E50 Ads.
Rovimix E50 Ads is 50% DL Alpha-Tocopheryl acetate. 500IU =500mg of DL Alpha Tocopherol. Hence 500IU is equivalent to 1g of Rovimix E50 Ads.
This was diluted with carrier so that it could be easily mixed with the feed in the trough. 1kg of the diluted mix contained 50 gram of Rovimix. i.e for the pen of 25 animals to be treated they received 0.5kg of the diluted mix per day.

Blood sampling

Pretreatment samples were collected just prior to the commencement of the trial on 11/2/94 and again on the 18/2/94.

Clinical examination of animals

The animals were clinically assessed prior to the commencement of the trial. Clinically these animals showed puffiness of the hock joint and lower leg associated with mild lameness. They were assessed again 10 days after the commencement of the trial.

Results:

The serum vitamin E levels before and after treatment are listed in Table 10.

Table 10: Response of serum vitamin E levels to oral administration of vitamin E

Animal Id	Serum vitamin E (μmoles/l) 11/2/94	Serum vitamin E (μmoles/l) 18/2/94	Change in Serum vitamin E (umoles/l)	Treatment
1	5.0	4.3	-0.7	NT
2	7.1	5.5	-1.6	NT
3	4.0	4.5	0.5	NT
4	8.0	6.3	-1.7	NT
5	3.9	3.5	-0.4	NT
6	5.9	4.8	-1.1	NT
	Mean= 5.6 (±1.7)	Mean= 4.8 (±1.0)	Mean= -0.8[a] (±0.8)	
7	4.1	4.5	0.4	T
8	3.4	4.8	1.4	T
9	3.0	5.9	2.9	T
10	4.8	7.6	2.8	T
11	3.6	4.1	0.5	T
12	5.8	7.1	1.3	T
	Mean= 4.1 (±1.0)	Mean= 5.7 (±1.4)	Mean= 1.5[b] (±1.1)	

An unpaired t-test was used to compare the change in serum vitamin E levels for the treated and untreated groups. The means with different superscripts are significantly different ($p<0.01$).

T = treatment -addition of 500IU Vitamin E per head per day from 11/2/94 Vitamin E supplied by Fred Schwenke of Colburn Dawes, Brisbane.

NT= No treatment - normal ration

( ) after mean = ± Standard deviation

Serum vitamin A levels were all normal and serum β-carotene levels were very low both before and after treatment.

After 10 days (21/2/94) reexamination of the Control group showed very little change in the swelling, whereas in the treatment group only 2 animals still had signs of leg swelling:- one was stiff, and another was a bit puffy in the lower legs and hock.

Conclusion: The addition of supplementary vitamin E at 500 IU/animal/day to the diet of animals with leg-swelling,

significantly increased the serum levels of vitamin E and alleviated the clinical signs of leg swelling in the majority of animals. The level of increase in serum levels was not great and did not approach levels normally seen in cattle on green feed. Hoppe *et al* (1993) demonstrated in pigs that plasma alpha tocopherol was related to the logarithm of supplemental dietary vitamin E.

Response to injectable vitamin E (Vitajec)

Individual animals with leg-swelling at feedlots 5,8,16,17 were injected with a single dose of Vitajec. In general there was no apparent beneficial effect.
This is not surprising since the level of vitamin E in Vitajec is very low at 100 IU/2ml dose.

4.5.8 Monitoring serum vitamin A and E after increasing dietary vitamin E from 160 to 320 IU per beast per day

At feedlot 5 in February 1993 the specified levels of vitamin supplementations were vitamin A at 45000 IU/animal/day and vitamin E at 160 IU/animal/day.

In May/June 1994 the level of vitamin E was increased to 320 IU vitamin E /animal/day. Vitamin A remained the same at 45000 IU/head/day.

Table 11: Serum vitamin E levels when supplementing at 160 and 320 IU vitamin E/head/day.

Days on feed	Date	Specified vitamin E levels in feed (IU/head/day)	Serum vitamin E (μmol/l)
150 (9 samples pooled)	17/2/93	160	7.76
150 (9 samples pooled)	"	"	6.87
180 (12 samples pooled)	"	"	7.01
150 (10 samples pooled)	29/7/94	360	8.24
250 (10 samples pooled)	"	"	7.20

The serum vitamin E levels have risen when supplementary vitamin E has been increased from 160 to 320 IU/head/day when comparing the levels in the groups of cattle fed for 150 days (Table 11). Although the levels have increased and have risen above levels which would be associated with clinical deficiency, they are not as high as levels attained with the feeding of fresh green fodder (usually between 15 and 25 μmol/l).

4.6 Possible consequences of vasculitis/microangiopathy of the limbs

4.6.1 Vascular leakage and fragility

In pigs and chickens vitamin E deficiency is associated with damage to small blood vessels resulting in the leakage of fluid from the vessels and the accumulation of oedema fluid around the leaking vessels. Vascular damage is not traditionally recognized as a clinical feature of vitamin E deficiency in ruminants but McMurray *et al* (1982) observed extensive vascular lesions in some cattle from a group in which they were experimentally inducing white muscle disease by feeding a selenium/vitamin E deficient diet and polyunsaturated fats. Because free-radical

damage affects cell membranes it is conceivable that these damaged vessels not only leak fluid through their walls but they may be more fragile and prone to rupture and haemorrhage.

4.6.2 Impaired thermoregulation and the effect of climatic conditions

The decline in Vitamin E status in the summer may be a reflection of higher requirements or deterioration of natural or supplementary vitamin E in the feed. The field observations of the feedlot veterinarians at feedlot 1 and 16 suggested that there was a relationship with weather patterns particularly to stormy hot humid weather of January. From the replies of the questionnaire no other feedlots had made this observation. A common observation by staff at the feedlots which were closely monitored was that the condition of leg swelling occurred during prolonged hot weather and subsided with the onset of cooler weather.

From the results of the questionnaire (ref. to Appendix 1) and the analysis of the replies from 15 feedlots, the months November, December, January, February and March were the most common months in which the condition occurred.
From the questionnaire the problem occurred in the warmer regions of Australia, and in general occurs mostly in hot weather.

Feedlot veterinarians reported that affected animals often had elevated rectal temperatures.

At feedlot 5, blood samples were collected and rectal temperatures measured from 8 affected animals. Rectal temperatures were also taken from 8 normal starter cattle at the same time and in the same environment. Starter cattle were used as controls because they would not have been influenced by dietary factors in the feedlot ration. It would have been best to have a second control group of animals at the same stage in the feedlot but without leg swelling, but this was not possible because of the management disruption caused by pulling cattle from their pens to the yards. The weather was overcast, humid, threatening thunderstorms, with the temperature in the mid 20Cs. The rectal temperatures were significantly higher in the affected cattle than in the control cattle.

Table 12: Rectal temperatures in cattle with leg swelling and unaffected cattle.

Swollen-leg cattle identity	Rectal temp. ^{o}C	Normal starter cattle identity	Rectal temp. ^{o}C
1a	-	1c	39.8
2a	40	2c	39.8
3a	39.2	3c	38.8
4a	40.3	4c	38.5
5a	40.1	5c	38.7
6a	40.7	6c	39.0
7a	38.4	7c	38.7
8a	39.8	8c	38.9
Mean ($\pm$ SD)	Mean= 39.8($\pm$ 0.76)		Mean=39.0 ($\pm$ 0.5)

Comment: Rectal temperatures are higher in the affected animals than in the controls These were statistically different when analysed by a t-test ($P< 0.05$)

Under conditions of high heat load, respiration is responsible for only about 15% of heat loss. The balance must be lost through the body surface either by conduction, convection, radiation and evaporation. This relies on transfer of heat from the body core and depends on the blood flow to the skin (Blackshaw and Blackshaw,1994)

Arteriovenous anastamoses (AVAs) are normal precapillary structures which connect the arterial and venous sides of the circulation thus allowing blood to bypass the capillary bed. They occur in the peripheral circulation of terrestrial and marine mammals and birds. These AVAs have an important role in heat dissipation. The large volume of warm arterial blood can be shunted quickly into superficial veins thus promoting heat loss. It is believed that they also maintain tissue viability when the extremities are exposed to a cold environment. By opening they can maintain the tissue at a viable temperature. Under heat stress conditions it has been demonstrated that approximately 70% of total limb blood flow in the sheep passes through AVAs (Hales 1985).

From the histopathological examination of affected legs it appears that these vessels are damaged and many are occluded. Without these vessels functioning properly it is conceivable that temperature regulation would be impaired. In addition the presence of subcutaneous oedema fluid would impair heat exchange. These vessels may be prone to oxidative damage because of their high metabolic activity and because they are anatomically located in the superficial tissues of the lower limbs. Membranes in these sites are most likely to contain high levels of PUFAs to maintain their fluidity at cold temperatures. Membranes with high PUFA levels are prone to oxidative damage. It is also conceivable that heat stressed animals may absorb higher levels of PUFAs. Ruminal pH is lowered in heat stressed cattle and ruminal and intestinal motility are reduced (Shearer and Beede, 1990) and as a result hydrogenation of ingested PUFAs may not be complete.

The affected animals had elevated rectal temperatures when compared with control cattle under the same environmental conditions. To determine definitely if thermoregulatory mechanisms were impaired, temperatures would need to be measured at different times of day and the environmental temperature monitored.

We have also observed that feedlot cattle deficient in both Vitamin A and Vitamin E have defective thermoregulatory mechanisms and are susceptible to heat stress (Refer to Appendix 2).

4.6.3 Feedlot cattle deaths from heat stress

During investigations into leg-swelling, two feedlots in northern NSW and one in southern Queensland suffered many deaths from heat stress during days of prolonged hot, humid weather from 9/2/91 until 13/2/91. The two feedlots in northern NSW had both had recent outbreaks of "leg-swelling" investigated by the Regional Veterinary Laboratory Armidale and the feedlot in Queensland had a condition of redness and swelling in the legs in November and December 1990 (Refer to Table 4, feedlots 1,8 and 16).

Death rates were from lowest to highest:-
30 out of 6000
91 out of 21000
2700 out of 17,000

A feature of these heat stress deaths was that while hot environmental conditions occurred throughout the region, deaths were confined to feedlot cattle and were not seen in grazing cattle. Possibly environmental factors such as lack of shade and crowding did not allow animals to dissipate heat quickly enough; alternatively the feedlot cattle were different physiologically. At the Queensland feedlot animals less than two months on feed were significantly less at risk, but there was no association with body weight and fat cover (Queensland DPI report). The same observation was made in cattle with leg-swelling. It would thus appear that deaths were associated with a physiological defect in heat dissipation.

The three feedlots (1, 8 and 16) which had heat stress related deaths were feeding either no supplementary vitamin E or very low levels (Refer to Table 4). In the early 1990s in Australia, there was a trend away from the use of vitamin E additives in feedlot rations because of a misapprehension that there was adequate natural vitamin E in the diet (Schwenke, 1992). From our investigations into leg-swelling, it appears that these recommendations were unsuitable for Australian summer conditions. Natural vitamin E in stored feed in silos is destroyed quickly and there is an apparent increased demand for vitamin E during hot weather.
For feedlot 16 high levels of vitamin A were included in the ration (See table 4). This high level of vitamin A would have decreased the level of vitamin E absorption.

Post-mortem liver samples were collected from 2 dead cattle from feedlot 8
The liver vitamin A levels were:- 132 mg/kg (460 umol/l) and 43.6 mg/kg (152 umol/l) These are normal and not deficient (unfortunately liver vitamin E analysis could not be done.)
Serum vitamin E levels were low in cattle with leg-swelling at feedlots 1 and 16 (Refer to Table 8) in samples collected just prior to the heat stress related deaths. The low levels of supplementary vitamin E at these 3 feedlots, the low serum levels and the occurrence of leg-swelling just prior to the heat stress deaths, suggest that vascular damage from vitamin E deficiency may have led to impaired temperature regulation.

4.7 Discussion

The proposed sequence of events initiating vascular damage and leakage in peripheral blood vessels particularly in the hind legs :-

Low levels of vitamin E in the body due to :-

- Low levels of supplementary vitamin E
- Low levels of the antioxidant B-carotene in the diet
- Grain based diets increase vitamin E requirements
- No storage organ for vitamin E (cf vitamin A storage in the liver), therefore tissue levels drop quickly when demand outweighs supply.
- Fat animals have high vitamin E requirements
- Imbalance between vitamin A and vitamin E supplementation leading to impaired absorption of vitamin E.
- Fat in the diet e.g. in tallow or whole cottonseed increasing the demand for vitamin E
- Dietary supplements such as ionophors (e.g. Monensin) increase the demand for vitamin E.
- Rancidity of feed which would diminish the level of vitamin E and other antioxidants in the feed.

Prolonged hot (and humid) weather :-

- Further diminishing tissue levels of vitamin E, because of increased demand and loss in feed
- Peripheral blood vessels more metabolically active and therefore more prone to oxidative damage.

Consequenses of damage to superficial vessels in the lower limbs :-

- Damage to the superficial vessels in the lower limbs leads to fluid swelling of legs and joints, redness of the skin, sometimes subcutaneous haemorrhage, inflammation and lameness.

- Because of the role of these vessels in temperature control, their damage could be a contributing factor in the inability to cope with heat stress with a resultant increased rectal temperature during hot weather.

- During extremely prolonged hot weather this inability to cope with heat stress may lead to death.

5 Success in acheiving objectives

In the main the objectives were acheived although the completion of the project was delayed. A group of researchers had been envisaged when the project proposal was made, but circumstances including a retirement, a redundancy and a relocation, left the principal researcher as the sole researcher in this project. The unforeseen financial squeeze on the Regional Veterinary Laboratory, Armidale by NSW Agriculture, re. the "freeze" on employment of extra staff during busy times or when staff were on leave, inhibited technical support for this project. The announcement by NSW Agriculture of the proposed and imminent closure of this laboratory has led to staff losses and cessation of technical support. Some electron microscopic studies were done, but could not be completed and have not been included in this report.

6 Impact on Meat and Livestock Industry

The findings from this project have been disseminated through the press to the feedlot industry. There has been a general trend to increase the supplementation of vitamin E because of the enhancement of meat keeping quality. The findings of this project will reinforce the need for added supplementation. It would appear that the incidence of leg swelling and deaths from heat stress have subsided over the last 2 summers. This may be related to higher levels of vitamin E being fed or may be related to seasonal variations.

7 Conclusions and Recommendations

7.1 Recommended levels of vitamin E and A supplementation

Levels of vitamin E supplementation recommended are :-

If no fresh green feed is included in the diet vitamin E supplementation is required.
0-60 days 150-200 IU per animal per day
60-300 days 200-500 IU per animal per day
In the warmer months of the year supplementation should be higher than in the cooler months.
Recent studies have shown beneficial effects on meat keeping quality of oral supplementation with 500 IU vitamin E per head/day. These higher levels have also been associated with a lowered incidence of infectious disease.

Refer to Table 13 - It is assumed that there is no fresh green feed included in the diet. These levels have been based on recommended levels in the literature as well as from the findings of this current study.

Table 13: Vitamin A and E recommendations for Australian feedlot cattle

TYPE OF ANIMAL	ANIMAL WEIGHT kg	VITAMIN A IU	VITAMIN E IU
CALVES	180	30,000	150-200
FINISHING	270	35,000	"
AS SHORT	360	40,000	"
YEARLINGS	450	50,000	"
FINISHING	270		200-500
	TO	50,000	"
YEARLINGS	500		"
FINISHING	360		200-500
	TO	50,000	"
2-YR.OLDS	550		"

7.2 Recommended ratio of vitamin A : vitamin E

In a well balanced ration the ratio of vitamin A : vitamin E should be about 400:1 or 200:1 or even lower but not higher than 1000:1. This is because excessive levels of vitamin A inhibit vitamin E absorption and vitamin E helps preserve vitamin A from oxidising.

7.3 Monitor the actual levels of vitamin E in the premix and/or ration

Discrepancies have been identified between the specified level of vitamin E and the actual level in the premix.(See Table 1, Feedlot 5). This is an area that requires further investigation. Vitamin E can be quickly depleted in the presence of minerals and alkaline pH. It is not uncommon for the vitamins, minerals and other ingredients including limestone (very alkaline) to be premixed and stored. The vitamin E may be losing potency under these conditions particularly in hot weather.

7.4 Monitor serum vitamin E and A levels

Serum levels of vitamin E can be used as an indicator of tissue levels. Abattoir samples from finisher cattle can be monitored at different times of the year. Because the test is expensive a number of samples can be pooled together. e.g. a pool of 10 sera from healthy finisher cattle. Refer to Appendix 3 -"Sample collection for vitamin A and E analysis" and Appendix 4 -"Bovine vitamin A and E levels."

7.5 Antioxidants in feed

e.g. Rendox and Endox (Kemin Aust. Pty. Ltd.) These may have a sparing effect on other antioxidants such as vitamin E and may be less expensive. This requires further investigation.

7.6 Seasonal requirements

From the findings of this study we have recommended that higher levels of vitamin E supplementation are given during the warmer months of the year (Refer to Section 7.1).

7.7 Costs

The price of vitamin E (dl tocopheryl acetate) is $60.82/kg pure.
(Rovimix E50ADS costs $30.42/kg, is 50% dl tocopheryl acetate - Colborn-Dawes Australia (Pty) Limited.
i.e. the price for supplementing one animal 300IU per day is 1.82c per day.

Hydrovit Oral ADE (Rhone-Poulanc Animal Nutrition)
Vitamin A - 100,000 IU/ml
Vitamin D - 10,000 IU/ml
Vitamin E - 40 IU/ml
1 litre - $29.50

Hydrovit E150(liquid)
vitamin E - 150 IU/ml
250 ml - $30.00, 1 litre - $69.65
i.e. 1.4c/300 IU vitamin E

Injectable vitamin E

Vitajec ADE injectable (Rhone-Poulenc Animal Nutrition) :-
Vitamin A - 500,000 IU/ml
Vitamin D - 75,000 IU/ml
Vitamin E - 50 IU/ml
100 ml pack -$17.55, 200 ml pack - $30.10, 500 ml pack - $38.85

Testing of vitamin A and E levels in serum, tissue, feed.

Laboratory	NSW Agriculture EMAI Biochemistry	Gerrard Smith WA Dept. Agriculture
Serum or plasma vitamin A and E together	$30.00	$6.00
Tissue or feed vitamin A and E together	$100.00	$14.00

7.8 Suggestions for future research

1. Measure serum vitamin E levels at different times of year as an indicator of varying needs according to the climate. Measure levels of natural vitamin E in stored feed at different times of the year to determine the depletion under Australian conditions.

2. To determine the effect of premixing vitamins with minerals and other feed additives on the level of vitamin E and the effect of storage of these premixes at different temperatures.

3. Rectal temperatures in relation to depleting vitamin E levels. Rectal temperatures measured at varying environmental temperatures. To ascertain if depleted vitamin E does in fact impair thermoregulation.

4. To determine the most cost effective method of vitamin E administration. Hidiroglou and Hidiroglou (1990) and Hidiroglou and Charmley (1990) investigated various experimental methods of administering vitamin E but these were not methods suitable for practical application. Gerrard Smith of the WA Department of Agriculture has found that the most effective method in sheep is the subcutaneous administration of an aqueous suspension of alpha-tocopherol acetate. This is superior to an oil suspension given intramuscularly and to oral administration (Smith *et al*, 1996).
   It would need to be determined if this was also the case in cattle and the optimum interval between injections would need to be determined. I have discussed this with Gerrard Smith and a joint cooperative project is envisaged.

5. Antioxidants in feed. Their cost effectiveness in sparing dietary vitamin E.

8 Bibliography

Refer to Appendix 8 for a full list of references

The Fat-Soluble Vitamins (1969) Eds. H.F.DeLuca and J.W.Suttie. The University of Wisconsin Press, Madison, Milwaukee, and London.

Veterinary Clinics of North America: Food Animal Practice - Vitamin deficiencies in cattle 7:217-275

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9 Acknowledgments

This project was supported by funds from the Meat Research Council.
I would like to acknowledge the support given by feedlot managers, their staff, feedlot veterinarians (Nigel Nichols, Bob Rheinberger, Bob Hunter and John Brown) DPIE veterinarians, Stephen Sinclair Regional Veterinary Laboratory Armidale, the staff in Biochemistry at the Elizabeth Macarthur Agricultural Centre, Camden, Ron Tume, Cannon Hill, the Beef CRC, and Terry McHugh, publicity officer, NSW Agriculture.

10. APPENDICES

APPENDIX 1

Questionnaire

Milestone 3.0 To establish the incidence of swollen hocks/swollen hind legs in Australian feedlots.

The following questionnaire was prepared for distribution to Australian feedlots registered with the Australian Lot Feeders Association. ALFA was approached regarding the distribution of the questionnaire (see Attachment 1). The questionnaires were distributed via a number of avenues.

i. Distributed to veterinarians and feedlotters at the ALFA/ELANCO workshop at Coffs Harbour in May 1994 Approximately 25 to vets. and 75 to feedlotters.
ii. Approximately 100 copies were sent with the proceedings of the workshop.
iii. Sent to members of the Australian Association of Cattle Veterinarians.
iv. Available at the NSW Agriculture stand at Beefex 94.
v. Sent to feedlots serviced by the Regional Veterinary Laboratory Armidale, NSW.

"SWOLLEN HOCK/SWOLLEN HINDLEG SYNDROME IN FEEDLOT CATTLE"

Research at the NSW Agriculture Regional Veterinary Laboratory, Armidale, into the cause of swelling of the lower legs in feedlot cattle has indicated that the condition may be related to low antioxidant levels, particularly vitamin E. Funding has been received from the Meat Research Corporation to expand these investigations with the ultimate aim of providing dietary recommendations to prevent the condition.

The following questionnaire has been designed to assess the extent of the problem in Australian feedlots and possible contributing factors in the diet.

IT WOULD BE OF GREATEST BENEFIT TO THIS SURVEY IF ALL FEEDLOTS ANSWERED THE QUESTIONNAIRE IRRESPECTIVE OF WHETHER OR NOT THIS PROBLEM HAS BEEN OBSERVED.

All information received will be treated as strictly confidential.

FEEDLOT NAME _____

FEEDLOT ID. (OFFICE USE ONLY) __

ADDRESS _____

POSTCODE ____

MANAGER _____

PHONE _____
FAX _____

NO. ANIMALS TURNED OFF PER MONTH ____

BREED (MARK Y OR N)

ANGUS OR ANGUS CROSS [ ]
MURRAY GREY OR CROSS [ ]
HEREFORD OR CROSS [ ]
OTHER BRITISH BREED (BOS TAURUS) [ ]
BOS INDICUS [ ]
BOS INDICUS CROSS [ ]

IS THE CONDITION SEEN [ ]

SEX OF AFFECTED CATTLE (MARK Y OR N)

MALE CASTRATE [ ]
MALE [ ]
FEMALE [ ]

EARLIEST AGE OF AFFECTED CATTLE (MONTHS) __

LATEST AGE OF AFFETCED CATTLE (MONTHS) ___

EARLIEST DAYS ON FEED OF AFFECTED CATTLE (DAYS) ___

LATEST DAYS ON FEED OF AFFECTED CATTLE (DAYS) ___

MONTHS IN WHICH CONDITION WAS OBSERVED (MARK Y OR N)

JANUARY [ ]
FEBRUARY [ ]
MARCH [ ]
APRIL [ ]
MAY [ ]
JUNE [ ]
JULY [ ]
AUGUST [ ]
SEPTEMBER [ ]
OCTOBER [ ]
NOVEMBER [ ]
DECEMBER [ ]

IS THERE AN ASSOCIATION WITH STORMY WEATHER

YES
NO
NO ASSOCIATION

LEVELS OF VITAMIN SUPPLEMENTATION IN THE DAILY DIET

VITAMIN A _____ THOUSAND IU PER HEAD PER DAY

VITAMIN E _____ IU PER HEAD PER DAY

OR

VITAMIN A _____ THOUSAND IU PER KG FEED

VITAMIN E _____ IU PER KG FEED

FEED INTAKE __ KG PER DAY

LEVELS OF VITAMIN A AND E INJECTED AT INDUCTION

VITAMIN A _____ THOUSAND IU PER ANIMAL

VITAMIN E _____ IU PER ANIMAL

ARE OTHER ANTIOXIDANTS (OTHER THAN VITAMIN E) USED IN THE FEED

YES
NO

IF YES, WHAT AND AT WHAT RATE

ARE OTHER FEED ADDITIVES INCLUDED E.G. MONENSIN ("RUMENSIN") AND LASALOCID ("BOVATEC")

YES
NO

IF YES WHAT IS USED _____

IS WHOLE COTTON-SEED, COTTON-SEED MEAL IN THE DIET

YES
NO

ARE VEGETABLE OILS OR LARD OR TALLOW IN THE DIET

YES
NO

DESCRIPTION OF THE CONDITION AND ANY OTHER COMMENTS :-

RESPONSES TO QUESTIONNAIRE

A total of **15** replies were received from feedlots and a number of replies were received from feedlot veterinarians. The results from the 15 feedlots have been analysed and are presented below. Not all questions have been answered. The number of replies to each question has been recorded. The replies from the feedlot veterinarians are presented later.

Replies by State

VIC 1

NSW 7

QLD 7

The one feedlot in Victoria has not seen the syndrome. They have fed cattle over the past 6 years at 240 to 320 days on feed.

No. of animals turned off per month (13 replies)

Range 50-4000

Mean 1449

Standard deviation 1391

Breed of cattle on feedlot:-

BREED	ANGUS OR CROSS	M.GREY OR CROSS	HERE. OR CROSS	OTHER BRIT.	BOS INDIC.	BOS INDIC. OR CROSS
Replies	14	14	14	14	13	8
Yes	11	10	10	6	6	2
No	3	4	4	8	7	6

The one feedlot with pure *Bos indicus* cattle refer to the condition as laminitis (sore feet, curled up toes) in cattle 40 days plus on feed. The condition is seen in high grade Brahman bulls.
The condition was seen in feedlots that had British and *Bos indicus* breeds.

Is the condition seen? (14 replies)
9 yes 5 no.

Sex of affected cattle on feedlot (11 replies)

	MALE CASTRATE	MALE	FEMALE
REPLIES	11	11	11
YES	10	1	2
NO	1	10	9

Earliest age of affected cattle (9 replies)

Range 10-24 months
Mean 16.9 months
Standard deviation 5 months

Latest age of affected cattle (7 replies)

Range 22-48 months
Mean 32.3 months
Standard deviation 8.75 months

Earliest days on feed (8 replies)

Range 30-100 days
Mean 60 days
Standard deviation 25.6 days

Latest days on feed (4 replies)

Range 50-300 days
Mean 160 days
Standard deviation 112.8 days

Months in which condition was observed (8 replies)

MONTH	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
YES	6	6	6	4	3	4	4	4	3	3	5	5
NO	2	2	2	4	5	4	4	4	5	5	3	3

One NSW feedlot observed the problem in June, July and August in cattle 30 to 90 days on feed.

November, December, January, February, and March were the most common months that the condition occurred.

There was no difference between NSW and Queensland re. the months of the year that the condition was seen.

Is the condition associated with stormy weather? (7 replies)

1 perhaps 6 no

Levels of vitamins A and E added to the diet

	VITAMIN A (IU PER HEAD PER DAY)	VITAMIN E (IU PER HEAD PER DAY)
MEAN	45,6OO	232.5
RANGE	28,000-66.500	24-911
SD	10,100	257.5
NO.OF REPLIES	10	10

There was no correlation for the levels of vitamin A fed and the occurrence of the condition.
There was a trend that the vitamin E supplementation was lower in feedlots that saw the condition.
Range 24-392 IU (mean 157, SD 118) vitamin E per beast per day in feedlots with the condition.
Range 133-911 IU (mean 232, SD 257) vitamin E per beast per day in feedlots that did not record the condition.

Feed intake (11 replies)
Range 9-15 kg
Mean 13 kg
Standard deviation 1.7 kg

Level of vitamin A injection at induction (10 replies)
6 feedlots injected with vitamin A, 4 did not.
Range 1 - 2.5 Million IU per animal
Mean 2.08 " " " "
Stand. dev. 0.58 " " " "
4 feedlots gave no injectable vitamin A at induction

Level of vitamin E injection at induction (10 replies)
6 feedlots injected with vitamin E, 4 did not.

Range 100-250 IU per animal
Mean 208 " " "
Stand.dev.58.5 " " "

4 feedlots gave no injectable vitamin E at induction.

Vitamin levels were given by two feedlots but have not been included in the results because they are at great variance with normal supplementation and I believe they must have stated incorrect levels:-

One feedlot said that they fed vitamin A at a rate of 6 million IU per kg feed and vitamin E at 40g per kg feed. Their feed intake was 14.5 kg per day. This would mean they were feeding 87,000,000 IU vitamin A per head per day. 40g vitamin E = 40,000 IU and therefore they would be feeding 580,000 IU Vitamin E per head per day. Both levels are exceedingly high.

Another feedlot said that they injected 3750 IU vitamin A and 1.562 IU vitamin E per animal at the time of induction. This would be an extremely low dose.

Are other antioxidants used in the feed? (11 replies)
3 yes 8 no

One used Rendox @ 0.6mg/kg finished feed. Incorporated in the premix. This feedlot (ID 6) has seen the problem but not to any extent for the last 6 years other than 3 animals at 80 d.o.f. in winter 1994.
One answered Selenium

Are other feed additives included eg Monensin and Lasalocid (10 replies)

All yes
There was no correlation of these feed additives with the occurrence of the condition.
4 feedlots indicated they used monensin. One stated they were to replace this with Posistac.

Is whole cotton-seed, cotton-seed meal in the diet? (12 replies)

7 yes 5 no
From this information there was no association with the presence or absence of cotton seed or cotton seed products in the diet. Two feedlots specified that they used whole cotton seed. Both of these feedlots have seen the swollen leg problem.

Are vegetable oils, lard or tallow in the diet? (12 replies)

2 yes 10 no
Both feedlots that incorporated oils or tallow in the diet have seen the condition.

GENERAL COMMENTS BY FEEDLOTS:-

A NSW feedlot (ID 1) had an outbreak of the condition in 1982-83 and Jan 1987 in cattle greater than 90 days on feed. In 1987 the problem resolved with rain after a prolonged dry period. The problem recurred in the summer of 1990-1991 with subcutaneous and periarticular swelling around hocks. As the condition advanced the swelling extended down to the fetlock and pastern. No forelimb involvement. Swelling appeared weight related. The feedlot of 24,000 cattle had an estimated 30% affected over the summer. No deaths occurred but there were major disruptions to management as animals were put in hospital pens and the condition caused delays in turning off cattle.

Their consulting veterinarian estimated that this cost the feedlot $100 per affected beast.

A second feedlot in the same region (no reply was received to the questionnaire) had an outbreak in the summer of 1990-91. 7000 head on feedlot with an estimated 40% affected.

A NSW feedlot (ID 2) Normally cattle that are doing very well.

A Queensland feedlot (ID 4) Feed 8-10% whole cotton seed. Monensin included in premix. Have not observed the problem at all this year but common in past years.

A Queensland feedlot. (ID 6) Build up of fluid in hind legs. Not detected for several years in our feedlot except in three 340kg live weight Murray Grey steers which had approximately 80 days on feed in early winter. My amateur diagnosis (feedlot manager):- dietary potassium had fallen below our normal level. Corrected this and no problem since.

A Queensland feedlot. (ID 9) The condition described here we call laminitis (sore feet, curled up toes) and is normally only seen in high grade Brahman cattle.

A Queensland feedlot. (ID 10) We have only just commenced operations. Have not seen the problem. No details of vitamin supplementation were given.

A Victorian feedlot (ID 11) has fed many cattle over the past 6 years between 240 and 320 days, but we have no experience with swollen hocks (or other metabolic problem).

A Queensland feedlot (ID 12):
Cattle have more room to walk about than cattle in pens observed by me at other feedlots. We also precondition our cattle for 10 days on feed and pasture before shutting up. We have been running a feedlot for over 5 years and have never experienced the problem.

A NSW feedlot (ID 14):
When riding the pens you will notice steers knuckling over, very swollen and red. Sometimes it can be front legs or back hocks, sometimes both. It seems to happen at any time whether the weather is wet or dry. Things will go for a while with nothing wrong, then it will flair up overnight for no reason.

A NSW feedlot producer at the ALFA meeting at Coffs Harbour said the condition of swollen hocks has been seen in feedlot cattle and also in Limousine cattle being prepared for shows. He felt it was related to the quality of grain.

Follow up letters were sent to the 9 feedlots which reported the condition in an attempt to determine the percentage of cattle affected in the feedlot during an outbreak. (See Attachment 2). Only 1 reply was received. This feedlot would have less than 2% affected per year. Feedlot 1 had already indicated that they had had 30% affected in the outbreak of 1990-91.

RESPONSES FROM FEEDLOT VETERINARIANS

An American feedlot consultant (veterinarian and nutritionist) currently based in Queensland also completed the questionnaire for 4 feedlots but did not identify the feedlots.

Breeds of cattle on feedlot
Angus or Angus cross
Murray Grey or cross
Hereford or cross

The problem was seen on the 4 feedlots

Male castrate

Age of affected cattle 12-24 months

Days on feed of affected cattle 60 days plus

Occurs in November, December, January, February
Primarily occurs in summer months when hot weather occurs.

Sometimes associated with stormy weather.

Vitamin A supplementation 40,000 IU per head per day
Vitamin E supplementation 20 IU per head per day
Feed intake 10 kg per day

No injection of vitamins A and E at induction ("Won't work")

Comments: Anasarca may be due to rancid tallow, fermentable feedstuffs, molasses, poor silage, distillers grains, brewers grains. May cause vitamin A deficiency due to free radical production inhibiting transfer across small intestine. Once symptoms develop, fails to respond to any therapy - injection or feed.

A Queensland veterinarian replied to the questionnaire:-

Although we have not experienced major problems of this syndrome we occasionally see individual animals. The cases are generally in cattle > 50 days on feed, almost always steers, generally British breeds or their cross. Treatment has included antibiotic therapy, anti-inflammatory therapy and hospital rest. Some cases respond, others do not. There does not appear to be a link to the time of year.

A NSW veterinarian rang regarding a Japanese owned feedlot with cattle up to 450 days on feed. Long term feedlot cattle had widespread oedema. (Comment by B.Vanselow - Most likely a vitamin A deficiency syndrome.)

A Queensland veterinarian previously based in NSW reported that he has seen puffy hocks which appears to be bulging of fluid in the joint capsule. He has observed this in feedlot cattle on grain usually greater than 45 days on feed. He has recently observed the same condition in young bulls being fed high quality forage oats and grass in Spring 1995. The animals are growing rapidly and he suggests that it may be related to stresses on the joint. There is no associated lameness or elevation in rectal temperature. He has seen it in feedlot cattle in stormy weather but has suggested that the wet boggy conditions in the pens are putting more strain on the hock joints because of the extra effort required to move about and because of the greater likelihood of slipping and sliding. He has also observed feedlot cattle with generalised swelling of the hind leg and says it has occurred as outbreaks sometimes in individual

pens.

A NSW veterinarian investigated 8 deaths in 45 Hereford feedlot cattle. Two years old 110 days on feed. Mainly sudden deaths but 2 were seen sick prior to death, both showed lameness and depression, with death soon after. Myoglobinuria and muscle necrosis. Diagnosis: Nutritional or toxic myopathy (Ref. AN94/3470)

A NSW veterinarian diagnosed White muscle in 18 month old Hereford steers, 90 days on feed. Puffy hocks, myoglobinuria. Post-mortem- Blood tinged gelatinous exudate in sub-lumbar region, white muscle lesions in myocardium. Some recovered when put on green feed. Diagnosis: Nutritional or toxic myopathy

SUMMARY

From the replies received from 15 feedlots, 9/14 feedlots have seen the condition. Assuming that at least 75 feedlots received the questionnaire, a minimum of 9/75 (i.e. 12%) have experienced this problem.
In an affected feedlot the incidence may be as low as <2% to 30-40%.

The condition has been seen in both sexes including entire and castrated males.

The condition can occur at any time of the year but predominantly in the summer months.

The condition is sporadic and unpredictable.

It occurs usually between 60 and 160 days on feed, but the earliest mentioned was 30 days on feed, and the latest 300 days on feed.

Two comments from veterinarians implicate rancid tallow.

Both feedlots feeding tallow had seen the problem as did both feedlots feeding whole cotton-seed (high oil content).

There is some indication that the condition is related to low vitamin E levels in the diet.

From comments from veterinarians, a number of conditions may be covered by the terms "swollen hock" "swollen legs". These include:-

i. The sudden appearance of reddening and subcutaneous swelling of hind legs, usually from hock to coronary band and swelling of hock joint. Cause unknown (The subject of this investigation).

ii. Vitamin A deficiency which progresses to a generalised swelling of all 4 legs, brisket and submandibular region.

iii. Weight related or traumatic arthritis of hock joint.

iv. Nutritional or toxic myopathy.

For this reason it is possible that the responses received from feedlots may have included conditions other than the one we wished to investigate. With vitamin A deficiency and myopathy other signs would be manifest as well as the leg swelling.

Only 10 of the 15 feedlots which replied to the questionnaire revealed the level of vitamins A and E in the ration or the levels of vitamins A and E given at induction.
Only 11 of the feedlots revealed their feed intake.

Because many feedlots treat the details of their rations as confidential there have been great limitations to the amount of information that could be gathered in relation to this problem.

The questionnaire preparation and analysis was done on Epi Info Version 5 distributed by:
USD, Incorporated
2075A West Park Place
Stone Mountain, GA 30087
USA

Attachment 1

Bob Coombs,
Executive Director,
Australian Lot Feeders' Association,
PO Box 286
Jamison Centre ACT 2614 23/11/93

Dear Mr Coombs,

In 1992 I applied to MRC for external funding to investigate the problem of swollen legs in feedlot cattle. I was grateful to ALFA for supporting my application which was successful. My project is entitled:- "Continued investigations into the pathogenisis of a new bovine disease, microangiopathy, related to low antioxidant levels in feedlot cattle" and funding commenced in July this year.

I would like to determine the extent of the problem in Australian feedlots and feel this would be acheived best through a questionnaire. Is it possible to obtain a mailing list of feedlots? If not would it be possible to have the questionnaires sent through ALFA with the costs of postage paid from the research grant.

Because the clinical condition has previously been observed only in the warmer months, I would like to post the questionnaires before the end of the year.

Thankyou again for your support.

Yours sincerely,

Barbara Vanselow BVSc PhD
Veterinary Research Officer

No reply was received to this letter. Permission was given by ALFA to distribute the questionnaires at The ALFA feedlot workshop at Coff's Harbour in May 1994.

Attachment 2

The following letter was sent to the 9 feedlots which said that they had seen the problem.

Re. questionnaire on "Swollen hock syndrome in feedlot cattle"

Thankyou for replying to the questionnaire.
I have received 15 replies with the "swollen leg" problem being recognised in 9 feedlots.

I would like to determine the incidence of the problem in these 9 feedlots. Could you estimate the total number of cattle showing the symptoms during an outbreak as a percentage of the total cattle population in the feedlot. It only needs to be a rough approximation. Any other comments on the condition would be appreciated.

Thankyou,
Yours sincerely,

Barbara Vanselow,

Only one reply was received. A NSW feedlot (ID 2) The feedlot would have less than 2% affected per year.

APPENDIX 2

The following paper was published in the Annual Proceedings of the Australian Society for Veterinary Pathology, March 1994.

HYPOVITAMINOSIS A AND E IN A CATTLE FEEDLOT

B.A.VANSELOW & R.J.HUNTER***

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PMB (UNE)
Armidale NSW 2351

***Glen Innes Veterinary Clinic*
233 Ferguson Street
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The association of clinical disease in grazing animals with a prolonged lack of fresh green feed is age old. Jeremiah Ch.14, verse 6 describes wild asses whose "eyes did fail because there was no grass".

In the feedlot situation, grain and silage based diets contain inadequate amounts of β-carotene [the precursor of vitamin A] and vitamin E to maintain rapidly growing animals, and therefore supplementation is essential. Synthetic vitamins A and E are routinely added to feedlot diets but some feeding regimes fattening cattle for the Japanese market do not include supplementation.

When grazing animals ingest green feed, the β-carotene is split into two molecules of vitamin A in the small intestine. The vitamin A is carried as retinol bound to the albumin fraction of lipoprotein in the blood stream and stored in the liver esterified to long chain fatty acids. Some β-carotene is also absorbed and has been shown to have antioxidant properties. Vitamin A is necessary for the development of epithelial cells, for normal bone growth, and for the production of visual purple [rhodopsin], the retinal pigment required for adaptation to poor light. Both vitamin A and β-carotene are easily oxidised and destroyed particularly in the presence of oxygen, heat, light and moisture. Vitamin A [whether stabilized or not] is more rapidly oxidised when trace minerals are mixed with it as a concentrate. Excessive nitrates, polyunsaturated fatty acids, deficiency of phosphorus, deficiency of vitamin E, infectious disease, parasitic infestations, and diarrhoea may all affect the availability of vitamin A from the intestine. Vitamin A storage is principally in the liver, and the level in blood changes little until the liver stores are virtually depleted and then a rapid fall in blood values occurs.

Vitamin E is an antioxidant produced by plants which is particularly abundant in the germinal layer seeds in association with their high content of polyunsaturated fatty acids [PUFAs]. Various formations exist but α-tocopherol is the most important biologically in mammals [Greek tocos=childbirth, phero= to bear - related to the early discovery of the role of vitamin E in foetal survival]. Prior to absorption, vitamin E is emulsified by bile in the small intestine. Pre-intestinal destruction can occur e.g. microbial action in the rumen, high levels of PUFA in the diet or absorption may be impaired e.g. in lipid malabsorption or in association with excess vitamin A in the diet. Little tissue storage occurs - the liver is not a specific storage organ as it is with vitamin A. Proportionally more vitamin E is present in fat than in other tissues. α-tocopherol fits into the membranes of organelles [microsomes, mitochondria] where it acts as a lipid soluble antioxidant protecting adjacent polyunsaturated phospholipids and sulphydryl groups that play a major role in maintaining membrane integrity and permeability. The vitamin E requirements of ruminants partly depends on the amount of PUFA in the diet. Unlike monogastric animals ruminants should absorb mainly saturated fats because ruminal microflora hydrogenate PUFAs. It has been demonstrated that the hydrogenation is not always complete and in situations of ruminal malfunction e.g. lactic acidosis, hydrogenation would not occur, and the unsaturated fatty acids would pass through the rumen and be available for absorption. PUFAs are easily oxidised. The resultant free radicals produced, may in turn oxidise

polyunsaturated phospholipids and sulphydryl groups in membranes causing membrane damage. Glutathione peroxidase is also an intracellular antioxidant but it acts in the cytoplasm while α-tocopherol acts in the membranes. In situations of marginal deficiency of either vitamin E or selenium, adequate levels of the other antioxidant will prevent disease. Gross deficiency of either antioxidant will result in oxidative damage. The clinical manifestations of vitamin E deficiency are related to membrane damage with the organs and tissues affected varying between species. This may be related to the genetic makeup of membranes and their susceptibility to oxidative damage.

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. It was estimated that approximately 2,000 cattle were affected at any one time (i.e. cattle between 220 and 300 days in the feedlot). The diet was based on grain and corn silage with no supplementation of vitamins A and E. At the time of entry each animal had been injected with 1,000,000 IU vitamin A and 100 IU vitamin E . In commercial injectable preparations the low level of vitamin E per dose is unlikely to be of therapeutic benefit and most likely included to prevent oxidation of the vitamin A.

The clinical condition became obvious in the summer months. Animals were noticed to be suffering from clear ocular and nasal discharges, swollen hind legs, varying degrees of lameness and inco-ordination, 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 ulcerations, 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 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.

Pathological findings consistent with vitamin A deficiency included squamous metaplasia of the parotid salivary duct and papilloedema of the optic nerve. Grossly and histopathologically oedema occurred in subcutaneous tissue, within muscle bundles and within nerve bundles. Microangiopathy was identified in association with and probably as a cause of the oedema. Microscopically there was damage to endothelial cells lining small blood vessels with subsequent leakage of fluid and protein into surrounding tissues. Blood vessels were affected in subcutaneous tissue, muscle and on the pleural surface of the lungs, diaphragm and chest wall. There was massive proliferation of small blood vessels. Endothelial cells were swollen, the lumen of many vessels was obliterated and thrombosis was observed. The blood vessel walls were oedematous. Hyaline degeneration was observed with a marked inflammatory response around vessels.

From severely affected animals both serum and liver levels of vitamin A and E were below normal. Serum levels of both vitamins were measured in relation to "days on feed". Both levels dropped with time, with vitamin A dropping to its critically low level at approximately 180 days and vitamin E not dropping to it's accepted low level. From liver samples collected at slaughter, vitamins A and E were deficient.
That is, vitamin A levels appeared to be critically depleted first with vitamin E levels becoming critically depleted in the clinically ill animals. CPK and LDH values were elevated in severely affected animals. Glutathione peroxidase levels were normal.

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 IU per head per day.

Vitamin A and E requirements are high for rapidly growing animals 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 and it is conceivable from our observations that either vitamin A or E or both are required for effective thermoregulation.

The vascular lesion 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) have observed a vascular lesion in Vitamin A deficient cattle. Vitamin E levels were not measured in these cattle. In human malnutrition, the disease Kwashiorkor 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 hypoalbuinaemia, 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 PUFAs or iron the condition worsens and the death rate rises. Both PUFAs and iron exacerbate oxidative damage (Golden and Ramdath,1987).

It has yet to be ascertained as to whether the microangiopathy and oedema seen in these feedlot cattle was due to low vitamin A or E, or β-carotene alone or in combination.

APPENDIX 3

SAMPLE COLLECTION FOR VITAMIN A AND E ANALYSIS

Serum or plasma - at least 2 ml, protected from light and heat. Can be stored frozen.

Liver - approx. 2 cm cube, protected from light and heat. Can be stored frozen.

Feed - Stored frozen, protected from heat and light.

[Note - these tests are expensive. Refer to section 6.7]

Note: An interesting finding at EMAI Biochemistry is that vitamin A and presumably other components of sunscreens, resist vigorous hand washing, and traces of vitamin A can contaminate the material used to filter extracts of the samples prior to injection into the HPLC. Gloves are now worn during the procedure.
Compare the vitamin A levels on duplicate samples with and without gloves.
There was no significant effect on vitamin E, although other hand lotions would affect it.

Sample	Vitamin A (μm/l) sunscreen, no gloves	Vitamin A (μm/l) gloves
1	2.1	0.35
2	2.4	1.61
3	5.1	1.79
4	3.3	0.74
5	2.7	2.00
6	2.9	1.12
7	2.3	1.40
8	2.3	0.84
Control 7	6.2	1.47

APPENDIX 4

BOVINE VITAMIN A & VITAMIN E LEVELS

	VITAMIN A (μmol/l)	**VITAMIN E** (μmol/l)
SERUM		
Deficient	<0.9	<4.6
Normal	0.9-2.1	4.6-20
	(μmol/g)	(μmol/g)
LIVER		
Deficient	<17	<11.6
Normal	20-1000	21-102

These levels are guidelines only. Other factors will influence the manifestation of disease.

APPENDIX 5

CONVERSION FACTORS FOR VITAMIN A AND E

VITAMIN A (MW 286.44)

SERUM/PLASMA

µg/ml x 1000/286.44 (=3.49) -> µmol/l
mg/l x 1000/286.44 (=3.49) -> µmol/l
mg/100ml x 10,000/286.44 (=34.9) -> µmol/l
µg/dl x 10/286.44 (=0.0349) -> µmol/l

TISSUES

µmol/kg x 286.44 -> µg/kg
µg/g (=ppm) x 1000/286.44 (=3.49) -> µmol/kg
µmol/kg x 286.44 x 1/0.3 (=954.8) -> IU/kg

VITAMIN E (MW 430.7)

SERUM/PLASMA

µg/ml x 1000/430.7 (=2.32) -> µmol/l
mg/l x 1000/430.7 (=2.32) -> µmol/l
mg/100ml x 10000/430.7 (=23.2) -> µmol/l
µg/dl x 10/430.7 (=0.0232) -> µmol/l

TISSUES

µmol/kg x 430.7 -> µg/kg
µg/g (=ppm) x 1000/430.7 (=2.32) -> µmol/kg
µmol/kg x 430.7 x 1/1000 (=0.4307) -> IU/kg

FEED

Vitamin A	Vitamin E
1 IU =0.3µg	1 IU = 1mg

APPENDIX 6

PUBLICITY

Preliminary data was presented in poster format at Beefex 1994.
Further results will be presented at Beefex 1996.

Response to press release, through NSW Agriculture, on vitamin E, heat stress and leg swelling in feedlot cattle. January 1996.

Radio interviews :-

With Linda Allen 2UE
With ABC Kempsey

Television news report :-

Prime TV

Newspapers :-

The Land - see attached press cutting
Nornews - see attached press cutting
Agriculture Today - see attached press cutting
Other regional papers
Breed Societies

Telephone calls :-

Alan Ward-Smithton (Fax 065 654476)
Kempsey dairy farmer, milks 100 cows. Fed 11kg/day grain, brewers grain, corn silage, mineral supplement, ADE injections. Run of hot weather signs of heat stress.

Charlie Bunce (Ph 521444)
Veterinarian Moree. Leg swelling in show cattle and feedlot cattle.

Barry Lamph (Ph 068 244372, Fax 068 244378)
Emu farmer "Longstowe" Warren 2824. 450 emu chicks on 10 acres. In hot weather show heat stress and feet swelling. Feed contains 16% protein, lupins, vitamin mix.

Keith Hutton (Coprice Feeds PO Box 561 Leeton 2705, Ph 069 530438)
Researcher in rice pollard which contains 20% PUFAs. Interested in the effect of rancidity.

Joy Haycock (Phone 068 464060, Fax 068 464179. Po Box 33, Yeoval NSW 2868)
Leg swelling in show cattle. Santa Gertrudis stud. Fed bull mix for 3 months. Leg swelling begins 2 months into feed. Heat related.

Chris Simmons (065 851626)

Dairy cattle with laminitis. Had spoken with Roger Cook at RVL Wollongbar. Group of 65 heifers fed Anionic Dry Cow Mix (ACDM - high sulphates to prevent milk fever) and were fed Dairy meal mix. The group that were given the ADCM developed acute laminitis and subsolar necrosis 10 days after calving. The group that were not given the ADCM were not affected. In the affected cattle muscle enzymes were elevated, temperatures elevated, animals eating well. No fluid retention. pasture - kikuyu, ryegrass, clover. ADCM - Ca, Ph, Mg, MgSO4, Vit, Monensin, protein meal. A lot of wet weather at the time.

Steve Collins ("Ben Eden" Delungra 2403 Fax 249155)

Feeds show bulls Jan/Feb develop leg swelling after approx 2 months. some grains worse than others. access to a small amount of green feed in paddocks. Weight of bulls approx. 750kg.

Rainer Reuss
c/o Dr Jim Desmarchelier
CSIRO Division of Entomology
GPO Box 1700 Canberra ACT 2601
Tel 062464001, Fax 062464000

Letter received requesting information on feed storage and vitamins.

Jack Sampson
Moyhu Vic 3732

Letter re. fresh green feed versus feedlot rations.

APPENDIX 7

LITERATURE REVIEW

OXIDATIVE DAMAGE WITH PARTICULAR REFERENCE TO VASCULAR PATHOLOGY AND THE ROLE OF ANTIOXIDANTS IN ITS PREVENTION.

CONTENTS

INTRODUCTION

Oxidation is the chemical process that causes metal to rust, fats to go rancid and foods to spoil. Historically the term oxidation referred only to when a substance combined with oxygen and in the process lost an electron. The term, oxidation, has since been broadened to include any process where a substance loses an electron or electrons as part of the chemical reaction known as oxidation-reduction: a process in which a substance loses electrons and another gains an equal number of electrons. The reaction is accelerated by exposure to moisture, heat, oxygen and metals. Can this reaction also occur in living tissue?

Because the conditions suitable for oxidation occur in living tissue it is not surprising that this reaction occurs constantly. If it were not for the protection afforded by a network of chemicals known as antioxidants unchecked oxidation would occur with devastating consequences. Numerous disease conditions of both animals and man are now attributed to an imbalance between these antioxidants and oxidation.

FREE RADICALS

Oxidation causes electron loss and the consequent production of substances known as free radicals. A free radical (or oxidant) is any molecule or molecular fragment containing one or more unpaired electrons, i.e. electrons present alone in atomic or molecular orbitals. The unpaired electron usually gives a considerable degree of chemical reactivity to the free radical in its quest to gain an electron or to shed the unpaired electron. In chemical formulas the unpaired electron is conventionally shown as a 'superscript dot', as with the hydroxyl free radical $OH^{\bullet}$.

Free radicals can be produced in living tissue by various processes and reactions. These can be divided into 2 main areas.

1. Exogenous (external) insults. Free radicals can form as a result of exposure to radiation: ionizing radiation, UV radiation, and visible light with photosensitisers, and can also be formed by toxins including bacterial toxins. The pathological consequences of many bacterial and viral infections involve oxidation.
2. Endogenous (internal) insults. Free radicals can form by intracellular reduction-oxidation (redox) reactions involving the transfer of an electron. These reactions can be catalysed by metallic ions, and by enzymes. A pathologically very important source of free radicals is activated neutrophils. These phagocytic cells are known to generate an array of free radicals once they have been activated. Excessive and chronic activation of neutrophils can lead to deterioration of the immune response, mutations and tissue destruction.

The resultant oxidation of living tissue can be potentially harmful and the resultant changes referred to as

oxidative damage.

Oxygen is potentially damaging to living tissue (Mills, 1995). The inherent instability of oxygen is utilised by the body in aerobic respiration to form adenosine triphosphate (ATP) but in this process of the stepwise reduction of oxygen to water, free radical oxygen species are generated which are capable of damaging DNA, proteins, carbohydrates, and lipids. These species of radicals include the superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{\bullet}$), and singlet molecular oxygen ($^1O_2$). Other intracellular enzyme reactions are also responsible for the production of these free radicals. In addition to the toxic potential of these free radicals, those produced through the oxidation of lipids are also toxic.

Figure 1: The stepwise reduction of oxygen to water in animal tissues with the resultant production of free radicals (Adapted from Ceolho,1991a, and Bus and Gibson,1979).

$O_2 + 1e$ ------> $O_2^{\bullet -}$

$O_2^{\bullet -} + 1e + 2H^+$ ------> H_2O_2

$H_2O_2 + 1e + H^+$ ------> H_3O_2 ------> $H_2O + OH^{\bullet}$

$OH^{\bullet} + 1e + H^+$ ------> H_2O

as well $O_2^{\bullet -} + H_2O_2$ ------> $OH^- + OH^{\bullet} + {}^1O_2$

Organisms that utilise aerobic metabolism were only able to evolve with the evolution of antioxidant defence systems. The higher forms of life, animals, synthesize their own antioxidants as well as harnessing chemicals such as vitamin E and carotenoids from ingested plant material.

The hydroxyl radical, $OH^{\bullet}$, is possibly the most reactive radical to form in living organisms. This radical is produced exogenously or endogenously. It can be produced by exposing water to high-energy ionising radiation, splitting the water molecule. Since most of a cell is water, irradiation of a cell produces $OH^{\bullet}$, which causes substantial damage. This radical can also be produced in the stepwise reduction of oxygen to water. Initially in this reduction reaction, the superoxide anion radical is formed followed by hydrogen peroxide and then in the presence of ferric ion, the hydroxyl radical (See Figure 1). Cells have mechanisms to scavenge the superoxide radical and hydrogen peroxide before the hydroxyl radical can form.

The high chemical reactivity of strong free radicals ensures that their half-lives in the liquid phase of biological materials are short. For example the half-life of the hydroxyl radical is probably less than 1 nanosecond (Slater *et al*,1987). For this reason, tissue damage occurs in close proximity to the site of free radical formation.

The hydroxyl radical will attack almost any biological molecule that it is generated next to. It will cause breaks in DNA, hydroxylate purine and pyramidine bases, and attack membrane lipids and proteins. Proteins, carbohydrates and lipids are all susceptible to damage but the radicals have a preference for polyunsaturated molecules and frequently fatty acid group molecules are attacked. The structure and function of membranes largely depend upon the presence of different classes of phospholipids with different types of fatty acids. Membrane bound lipids are particularly susceptible to oxidation because they have an abundance of unsaturated fatty acids. Generally speaking, the most extensive changes in polyunsatureated fatty acids (PUFAs) that occur during peroxidation of biomembranes involve the highly unsaturated acids $C_{20:4}$ (arachidonate) and $C_{22:6}$ (Docosahexaenoate) (Slater *et al*, 1987). Unsaturated fatty acids are susceptible to oxidation since the presence of a double bond weakens the carbon-hydrogen bond of the carbon atom adjacent to the unsaturated carbon-carbon bond allowing the hydrogen to be removed easily by a free radical. If free radicals, particularly $OH^{\bullet}$, form close to a membrane, they will attack the PUFA side chains in the membrane lipid. A potentially devastating chain reaction known as lipid peroxidation can occur if oxidation is allowed to continue unchecked. Free radicals can initiate lipid peroxidation by "activating" and removing the hydrogen from the carbon-hydrogen bond in the PUFA, to form water. The initiation of lipid peroxidation in biological tissues may be accomplished by singlet oxygen (a higher energy state of oxygen),

hydroxyl radicals, and a number of other radicals produced *in vivo* from a variety of environmental chemicals but it cannot be initiated directly by weaker radicals such as the superoxide anion radical and hydrogen peroxide.. The fatty acid radical left behind is unstable and a chain reaction of oxidation is propagated resulting in the formation of lipid peroxides. Lipid peroxides can in turn be converted to lipid peroxy radicals ($LOO^{\bullet}$), which are highly reactive oxidising agents. These attract H atoms to become lipid hydroperoxides (LOOH). This conversion can be catalysed by the presence of metallic ions such as iron (Bus and Gibson,1979).

Lipid peroxidation diminishes membrane fluidity, increases non-specific permeability of ions (e.g. Ca^{2+}) and may in-activate membrane-bound enzymes (Halliwell, 1987) and may cause oxidation of membrane proteins (Coelho,1991a). A lipid peroxide is more polar than anything that should be present in the hydrophobic interior of a biological membrane and thus alters the functioning of the membrane. In addition, hydroperoxides can break down to toxic chemicals such as aldehydes and ketones.

Peroxidation of unsaturated membrane lipids has far greater biochemical effects than mere deterioration of lipids. The intimate juxtaposition of unsaturated lipids and proteins in membranes makes the proteins susceptible to oxidation by lipid peroxy radicals resulting in lipid-protein and protein-protein cross-linking (Bus and Gibson,1979). In addition several membrane proteins may derive some of their structure from closely associated membrane lipids. The membranes of the mitochondria and endoplasmic reticulum have a high proportion of unsaturated fatty acids and consequently are highly susceptible to lipid peroxidative damage. Lipid peroxidation damage of mitochondrial membranes has been demonstrated to correlate with swelling and lysis of the mitochondria. Microsomal membrane enzyme activity is also affected by lipid peroxidation as exemplified in carbon tetrachloride poisoning. Other examples of cell injury associated with lipid peroxidation include erythrocyte haemolysis in vitamin E deficiency and breakdown of lysosomal membranes with resultant intracellular release of proteolytic enzymes (Bus and Gibson,1979) and the release of intracellular enzymes from muscle cells in myopathies associated with deficiency of selenium and/or vitamin E.

Aerobic organisms have evolved specific chemical defence mechanisms: the antioxidants. In addition to these, more general defence mechanisms exist. For example plasma contains numerous proteins which will bind metallic ions thus preventing them from catalysing oxidation reactions. The most general defence of aerobic organisms against lipid peroxidation lies in the structural characteristics of cell membranes. Membranes consist of a hydrophobic midzone between two hydrophilic surfaces. The hydrophobic midzone appears to be richly penetrated by proteins. The unsaturated lipids are found for the most part in the hydrophobic inner layer. As many free radicals are cations and anions, they do not readily penetrate into the hydrophobic layer to the unsaturated fats. Furthermore the close juxtaposition of proteins and saturated lipids hinders free radical reactions. The proteins provide a spatial separation for the unsaturated lipids and thereby can prevent the spread of radical chain reactions through the membrane. (Bus and Gibson,1979).

Despite the very harmful effects of free radicals, living organisms have harnessed their power for beneficial purposes. There are many examples of free radicals that have been put to a useful purpose in biological systems. Free radicals are produced by activated macrophages and granulocytes to kill invading organisms. Oxidative agents such as hydrogen peroxide, myeloperoxides and superoxides are released extracellularly or within phagosomes in a controlled response. Free radicals are thought to be important in cell communication. They have also been found to be important in the chemistry of various enzymes (Halliwell, 1987). The enzyme-catalysed oxidation of arachidonic acid to a hydroperoxide precursor of prostaglandins is an essential step of normal metabolism in mammals. Free radicals are necessary in electron transport in mitochondrial respiration.

ANTIOXIDANTS

The antioxidants are diverse (Forsyth and Guilford, 1995) and include vitamin E, vitamin C (ascorbate), carotenoids (including β-carotene and lycopene), flavenoids, bile pigments, urate and the enzymes: superoxide dismutase (SOD), glutathione peroxidase (GSHPX), catalase and ubiquinol-10 (the reduced form of coenzyme Q_{10}). In animals, the fat-soluble antioxidants carotenoids and tocopherols (vitamin E), cannot be synthesised but are absorbed from ingested plants. Water-soluble antioxidants include ascorbate (vitamin C) and thiols. Man is one of the few species unable to synthesise vitamin C.

The diverse nature of antioxidants allows them to provide lines of defence against oxidation at different stages of the chemical production of free radicals and at different sites. For example enzymes such as superoxide dismutase, catalase and glutathione peroxidase are water soluble and located in aqueous compartments of the cell: the cytoplasm and mitochondrial matrix, where they detoxify the superoxide radical and hydrogen peroxide. Vitamin E is fat soluble and is a membrane-bound antioxidant.

Oxidative damage occurs when there is an imbalance between reactive oxygen species and antioxidants (Di Mascio *et al*, 1991). In general, antioxidants function by providing an electron, usually with hydrogen, to free radicals.

Figure 2: The levels at which the enzyme antioxidants function (From Coelho,1991a and Hoekstra, 1975).

$$2H_2O_2 \xrightarrow{\text{catalases}} O_2 + 2H_2O$$

$$LOOH \xrightarrow{\text{peroxidases}} LOH + H_2O$$

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Glutathione peroxidase (SeGSHPX)

This enzyme which contains selenium converts peroxides to alcohols and acts in the cytoplasm of cells. It reduces the amount of peroxides, including hydrogen peroxide and lipid hydroperoxides, thereby reducing the amount of vitamin E required to maintain membrane integrity. Because this enzyme is present in the cytoplasm, it is believed that it has little access to lipid hydroperoxides formed within cell membranes and its main function is in the removal of hydrogen peroxide formed in the cytoplasm (Bus and Gibson, 1979). In converting the peroxide into an alcohol, the glutathione is changed from its reduced state to an oxidised state. The enzyme, glutathione reductase, allows a constant replenishment of reduced glutathione.

Superoxide dismutases

Superoxide dismutases (SODs) are enzymes required in the tissues of all animals for the detoxification of the superoxide radical. A copper/zinc-containing dismutase is found in the cell cytoplasm and a manganese-containing dismutase in the mitochondrion. These enzymes can reduce the concentration of superoxide in the cell but, in so doing, produce potentially toxic hydrogen peroxide which has to be removed by the peroxidases e.g.GSHPX (Arthur,1982). In mammalian tissue SOD is mainly an intracellular enzyme. Only small amounts are present in extracellular fluids such as plasma, synovial fluid or cerebrospinal fluid (Halliwell,1987).

Catalase

Catalase is located in peroxisomes and reduces hydrogen peroxide to water (Halliwell,1987).

Carotenoids

Carotenoids are widely distributed in nature, where they play an important role in protecting cells and organisms. Carotenoids protect plants against photosensitisation induced by their own chlorophyll and have been used in treatment of photosensitivity diseases. Carotenoids such as lycopene and β-carotene can inactivate (quench) free radicals such as singlet oxygen (Krinsky,1993). Singlet oxygen is generated by photochemical reactions or enzymatically or by the process of lipid peroxidation of biomembranes. β-carotene has the ability to inactivate peroxy radicals at low oxygen pressure (Rice and Kennedy, 1988). Carotenoids are believed to have a role in the prevention of cancer (Di Mascio *et al*,1991).

Bile pigments

The bile pigments bilirubin and biliverdin are believed to be antioxidants of physiological importance because of their ability to quench singlet oxygen (Di Mascio *et al*, 1991).

Vitamin C

Vitamin C is also a powerful antioxidant at the cell level. It acts as a scavenger of oxidising agents and is involved in regenerating vitamin E after it has been oxidized. Vitamin C is a water soluble vitamin synthesized from carbohydrates in plants. Most animals can also synthesize vitamin C in the liver via the glucuronic pathway. Its production is inhibited by deficiencies of vitamin E, vitamin A and biotin (Jaffe,1984). Vitamin C is important particularly as an antioxidant in blood plasma.

Vitamin E

The term vitamin E is now used for 8 substances which occur naturally in plants:-
Tocopherols - alpha, beta, gamma and delta.
Tocotrienols - alpha, beta, gamma and delta.

Vitamin E has a primary role in plants to act as an antioxidant, protecting polyunsaturated fatty acids within the plant from auto-oxidation.

Alpha-tocopherol has the greatest biological activity in animals.[Greek tocos=childbirth, phero=to bear - related to the early discovery of the role of vitamin E in foetal survival].

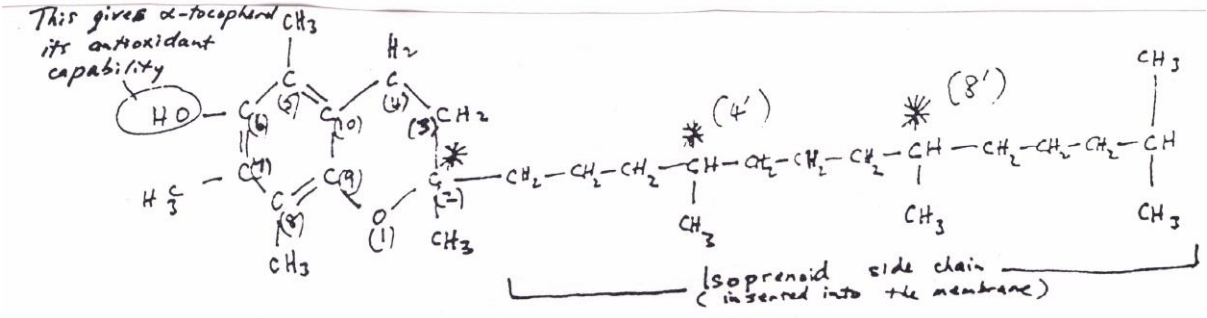
The biochemistry of alpha-tocopherol and its available forms

To understand vitamin E, the basic biochemistry must be understood. Figure 3 depicts the structure of alpha tocopherol. "α" refers to the number of methyl groups on the first ring (the chromanol ring). α-tocopherol has 3 methyl groups, while "β" and "γ" tocopherol each have 2, and "δ" tocopherol has one. The more methyl groups, the greater the antioxidant capability (Patton, 1989). The most important structural feature of vitamin E is the hydroxyl group on the chromanol ring which confers its activity as a biological antioxidant. Tocopherol can donate a phenolic hydrogen to the initial radicals formed during lipid peroxidation. The tocotrienols have only 16% activity compared with the tocopherols.

The phytol side chain forms an integral part of the membrane conferring stability on the membrane. The side chain is embedded into the membrane and the chromanol ring is at the surface of the membrane (Rice and Kennedy,1988).

The vitamin E activities of the tocopherols and tocotrienols are reviewed by Lynch (1991b). However, irrespective of their relative activity as antioxidants and of the concentrations of these various tocols in feedstuffs, only α-tocopherol is found in significant concentrations in the blood of pigs and cattle (Rice and Kennedy,1988).

Figure 3: Structure of alpha-tocopherol



With compounds such as α-tocopherol, various isomers are structurally possible because of the various possible combinations in relation to the spatial arrangement of groups bonded around 3 of the carbon atoms (marked on figure 3 with an asterisk). Each of the 3 carbons allows for 2 configurations, right (R from rectus, Latin for right)

and left (S from sinister, Latin for left) thus allowing for 8 possible stereoisomers of alpha-tocopherol (Figure 4). Synthetic vitamin E contains all 8 isomers and technically is called "all-rac", meaning it is composed of equal portions of all 8 isomers, or is racemic. Synthetic vitamin E is often referred to as DL-α-tocopheryl acetate referring to the left and right images. This terminology is misleading because there are actually 8 structural possibilities. The natural form in plants is 100% RRR.

Because the stereoisomers vary in their biological activity, allowances must be made when feeding synthetic vitamin E for the fact that 7 of the isomers are not as biologicaly active as the "natural" isomer.

The standard is 1 International Unit = 1mg of all rac (dl) alpha tocopherol acetate.

Figure 4 : The 8 possible stereoisomers of α-tocopherol that are possible because of the 3 asymmetric carbon atoms marked with an asterisk in figure 3. (From Patton, 1989).

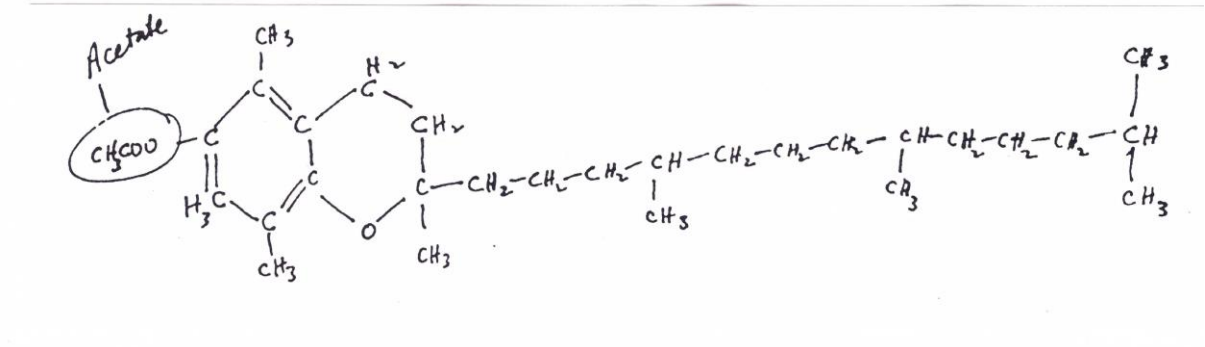
2R 4'R 8'R This form found in plants and called "natural".

2S 4'R 8'R These forms not found in forage, but in synthetic vitamins.
2R 4'S 8'R
2R 4'R 8'S
2R 4'S 8'S
2S 4'S 8'R
2S 4'R 8'S
2S 4'S 8'S

Natural vitamin E is unstable and quickly depletes in stored food (Patton 1989). Green forages, especially lucerne, are very good sources of vitamin E but vitamin E activity depletes with storage (Lynch, 1991a). Natural vitamin E, α-tocopherol, loses activity quickly when exposed to heat, oxygen, moisture, trace minerals and polyunsaturated fats in the feed. Natural vitamin E is such an unstable chemical, it should not be considered as a supplement in production-animal nutrition (Coelho,1991d).

Synthetic vitamin E has acetate attached to the hydroxyl group at C-6. (Adding the acetate invokes the nomenclature rule of changing tocopherol's "ol" ending to "yl".) This hydroxyl group is known to be crucial to the bioavailability of the vitamin and also known to be unstable. The acetate protects the molecule of vitamin E from oxidation. The synthetic vitamin E, tocopheryl acetate, is a weak antioxidant and will not act as such in premixes or feed. It becomes an antioxidant only after ingestion and hydrolysis in the intestinal tract but oxidation can occur particularly in concentrate food where oxidation increases following grinding, mixing with minerals, following the addition of fat and pelleting (Coelho,1991d). The stability of vitamin E acetate in premixes and feeds is determined by the particle size, and by the pH and mineral content of the premix or feed. Commercial vitamin E products are discussed by Lynch,1991d and Coelho,1991b,c.

Figure 5: Structure of alpha-tocopheryl acetate



Functions of vitamin E

Tissue antioxidant

Tocopherols are located almost exclusively in hydrophobic environments of membranes and in lipid-storage cells. Tocopherols, resident in biological membranes, are thought to be important in protecting the membrane-PUFA's from peroxidative degradation. Tocopherol performs that function by scavenging free radicals i.e. it donates them a hydrogen atom. It is thought that this oxidised tocopherol (tocopheroxyl radical) is recycled back to the metabolically active form by a reductive step that involves ascorbic acid. Otherwise the instability of the tocopheroxyl radical results in its spontaneous and irreversible conversion to tocopheryl quinone which is metabolically inactive and is excreted in the bile.

The most important structural feature of vitamin E is the hydroxyl group on the chromanol ring which confers its activity as a biological antioxidant. Tocopherol can donate a phenolic hydrogen to the free radicals formed during lipid peroxidation and thus control lipid peroxidation at a number of crucial points in the pathway.

Most of the α-tocopherol in animal tissues is located in the highly unsaturated subcellular fractions of cell membranes. These membranes are mainly plasma membranes and those of mitochondria and endoplasmic reticulum. Alpha-tocopherol is able to be situated in these membranes because it is lipophilic and its 16-carbon phytol chain confers on it the ability to form an integral part of the membrane. It may thus perform its free-radical scavenging function in close apposition to the peroxidising PUFA in the membrane (Rice and Kennedy,1988). Alpha tocopherol is incorporated close to enzyme systems such as NADPH oxidase which generate free radicals in the cell. Despite this unique activity, the amount of vitamin E in natural membranes is extremely low in relation to membrane phospholipid. The ratio of vitamin E to phospholipids is 0.05 mol per 100 mol or lower. Thus a minute amount of vitamin E exerts important and significant effects in protecting membranes against peroxidation (Packer and Kagen,1993).

Immune system

The role of vitamin E in immune responses has been reviewed by Sheffy and Williams (1980), Tengerdy (1988) and Finch and Turner (1996).

Vitamin E aids the immune response by:-
Stimulating glutathione peroxidise activity of circulating neutrophils, peritoneal macrophages and pulmonary alveolar macrophages and also stimulates T helper cells. Vitamin E also increases phagocytosis, antibody production (Ritacco *et al*,1986), and immune protection against several antigens and *Escherichia coli* (Barbar *et al*, 1977; Heinzerling *et al* ,1974). Since mitochondria and microsomes (ribosomes etc) act to produce antibodies and other defense mechanisms, it is clear that adequate vitamin E (and selenium) nutrition is important not only for prevention of overt signs of deficiency, but also for preservation of the organelles responsible for building the defense mechanisms against disease, radiation and other stresses.

Alpha-tocopherol and selenium have interactive beneficial effects on lymphocyte responses to antigen in *in vitro* assays (Pollock *et al*,1994).

Vitamin E has been found to be immunostimulatory for calves (Cipriano *et al* 1982, Reddy *et al* 1987) and dairy cows (Politis *et al* 1995).

Cellular respiration

Vitamin E is essential to cellular respiration particularly in heart and skeletal muscle.

Synthesis of coenzyme Q and vitamin C

Vitamin E may act as a cofactor in the synthesis of vitamin C and may stimulate the synthesis of coenzyme Q.

Inhibitor of platelet aggregation

Vitamin E is an inhibitor of platelet aggregation by inhibiting peroxidation of arachidonic acid, which is required for the formation of prostaglandins involved in platelet aggregation.

Interactions with vitamin A and β-carotene

Vitamin E improves the utilisation of vitamin A by facilitating the absorption of vitamin A and carotene and their storage in the liver. Excessive ingestion of vitamin A or carotene increases the requirement for vitamin E, whereas excessive vitamin E in the diet depletes vitamin A reserves. Therefore vitamins A and E need to be given in appropriate ratios (Hidiroglou and Williams, 1986).

Meat preservation

The deterioration of stored meat occurs because of lipid oxidation and muscle-pigment oxidation, causing a colour change (Smith *et al*,1994). The fat in meat is susceptible to autooxidation with the degree of oxidation influenced by several factors such as environmental temperature, presence of metals, enzymes, the type of fat and the levels of antioxidants. Hydroperoxides are produced, however at ambient temperatures they break down and produce a variety of hydrocarbons, aldehydes, ketones and alcohols. The production of these end products is the chemical manifestation of rancidity. The undesirable flavours in rancid materials can be caused by very small quantities of only a few p.p.m. of aldehydes and ketones. Therefore, the amount of lipid is actually less important than its susceptibility to oxidation.

The storage life of meat and its colour have been improved by the addition of vitamin E to the diet (Faustman *et al, 1989*, Lanari *et al*, 1993). Beef begins to lose its bright cherry-red colour as it is exposed to oxygen. It has been determined that in cattle the addition of vitamin E at 500IU per head per day during the last 100 days of the finishing period produces meat that stays fresh for up to 5 days longer. In the U.S. the cost of this feeding for 100 days is $1.50 per head (Smith,1994). Arnold *et al* (1993) demonstrated that vitamin E supplementation for at least 44 days at 1300 IU/day would produce beef with extended colour and lipid stability.

Mistumoto *et al* (1991a) demonstrated that the addition of vitamins E and C to ground beef lowered lipid oxidation and maintained the desirable colour of the meat. Mitsumoto *et al* (1991b) demonstrated that dietary vitamin E supplementation at a rate of 1200 I.U. per animal daily for 38 to 67 days prior to slaughter enhanced the meat keeping qualities. The oxidation of meat is reported to be initiated in the phospholipid-rich membranes (Buckley *et al*, 1989) and hence the incorporation of dietary vitamin E into the membranes may help prevent the initiation of oxidation. Dunlop and Powell (1994) investigated improvements in shelf-life of beef with vitamin e supplementation.

Absorption and storage of vitamin E

Vitamin E is absorbed in the small intestine and being fat soluble this is linked to fat absorption and requires the presence of bile salts and pancreatic enzymes for the formation of micelles. It is believed that the micelles contact the brush borders of the villi in the small intestine and the fat soluble components pass into the lipid phase of the cell membrane. Low fat diets and conditions that affect the pancreas and liver will diminish vitamin E absorption. The ester is hydrolysed in the gut wall, and thus the vitamin E is absorbed as the alcohol and transported via lymph to the general circulation. In the plasma it is mainly attached to lipoproteins in the globulin fraction. It is mainly found in the high and low density lipoproteins (HDL and LDL).

The enteric absorption of vitamin E is incomplete. Studies in rats and humans, have shown that only 20-30% of the vitamin is absorbed. A ten-fold increase in dietary intake of vitamin E is necessary to double the

concentration of vitamin E in the plasma.

It is believed that tocopherol reaching the peripheral tissues in lipoproteins is taken up following the recognition and uptake of those particles by specific receptors on cell surfaces. There is great variation between tissues on the amount of tocopherol taken up. Tocopherol is distributed strictly in the hydrophobic regions of cells, i.e. within the membranes and if any, the bulk lipid. Adipose tissue can account for as much as 90% of the vitamin E in the body, with liver and skeletal muscle accounting for most of the balance.

Tissues vary considerably in the rates at which tocopherol is depleted when the dietary intake of the vitamin is low. Adipose tissue, quantatively the most important site of tocopherol deposition, is mobilised at extremely slow rates and therefore poorly accessible to meet metabolic needs beyond that site. It is presumed that tocopherol functions in the lipid storage of cells as a chemical antioxidant to protect the polyunsaturated fatty acids stored there.

The absorption, transport and distribution of vitamin E have been reviewed by Bjorneboe *et al* (1989).

Plasma concentration of vitamin E

There is a relatively high correlation between plasma and liver levels of α-tocopherol, and plasma α-tocopherol concentrations can be effectively used to appraise vitamin E status (Ullrey,1981, Hoppe *et al*, 1993).

Optimal plasma concentration of α-tocopherol is usually considered to be at least 0.5 mg/100ml (11.6 μmol/l) as that concentration is associated with protection against *in vitro* erythrocyte haemolysis in mammals. Because the concentration is related to the lipid content it has been suggested that plasma tocopherol concentration should be expressed in terms of the lipid content. Since vitamin E is not specifically stored in tissues for metabolic accessibility, plasma or serum vitamin E levels can be utilised to assess the current status.

Serum α-tocopherol concentrations less than 200 μg/dl (4.6 μmol/l) are considered to be diagnostic of vitamin deficiency in cattle and sheep (Hoelscher,1978, Blood *et al*, 1989). Adams (1982) states that cattle with tocopherol concentrations of 0.3 mg/100ml (7.0 μmol/l) plasma or less have a marginal or deficient vitamin E status and that it would appear that cattle with 0.2 mg/100ml (4.6 μmol/l) α-tocopherol should be considered clinically deficient.

Alpha-tocopherol levels in plasma of less than 0.2 mg/l (4.6 μmol/l) are indicative of extremely low intakes. This is in contrast to values of 3.0-5.0 mg/l (7.0 - 11.6 μmol/l) or more found in young ruminants feeding on silage or grass which contains large amounts of α-tocopherol (McMurray *et al*,1983).

Factors increasing an animal's need for vitamin E (Adapted from Nockels quoted by Eng,1990)

Dietary

Nitrites - oxidising agents (Tengerdy, 1988)
High Vitamin A
High polyunsaturated fatty acids. PUFAs oxidise natural vitamin E in the diet and intestine and also increase the vitamin requirement in the body. An increase of 3 I.U. dietary vitamin E is necessary per each gram of PUFA (Putnam and Comben,1987; Coelho,1991e).
Linoleic acid may cause reduced absorption of vitamin E due to interference with micelle formation (Hoppe, 1990).
Propionic acid or sodium hydroxide preservatives (used to preserve grain)
High grain diets for ruminants
Chemical form of vitamin E
Low levels of other antioxidants
Low levels of copper, manganese, zinc, selenium, iron
Excessive levels of iron, copper
Ionophores (Tengerdy,1988)

Physiological

Rapid growth
Stress (Frye *et al* 1991) including heat stress (Williams, 1995)
Exercise (Jackson,1987)
Pregnancy
Lactation
Adipose tissue
Low vitamin C - although most animals synthesise ascorbic acid it can be low in young animals or during hypoglycaemia.

Pathological

Endotoxins (e.g. from *E.coli*) consume vitamin E
Ozone (a much more powerful oxidising agent than oxygen).
Aflatoxins
Gossypol in cottonseed (Lane and Stuart,1990)
Poisons (Bus and Gibson,1979)
Diarrhoea
Excessive production of neutrophils

Feeding recommendations

These recommendations depend on the required objectives of growth rate, immune response, serum level, tissue level and shelf-life of meat. The requirements will also depend on the challenge faced e.g. the amount of PUFAs in the diet, the amount of selenium, stress, infectious organisms and toxins.

ANTIOXIDANTS IN BLOOD PLASMA

Blood plasma contains an array of nonenzymatic low-molecular-weight antioxidants. These antioxidants can be classified into two groups: the water-soluble antioxidants present in the aqueous phase of plasma and the lipid-soluble antioxidants associated with lipoproteins. The water soluble antioxidants include protein thiols, uric acid, ascorbic acid and bilirubin. The lipid soluble antioxidants include α-tocopherol, ubiquinol-10, lycopene, and β-carotene.

Experimental evidence suggests that α-tocopherol is not of primary importance in protecting lipids in human blood plasma and low density lipoproteins (LDL's) against peroxidative damage. Quantitatively α-tocopherol plays a minor role in the protection of plasma lipids against water-soluble oxidants because relatively high concentrations of a number of natural antioxidants are present in the aqueous phase of plasma (ascorbic acid is particularly important). These water-soluble antioxidants also supersede α-tocopherol in qualitative importance in protecting plasma lipids against aqueous oxidants, presumably because of the physical proximity of the water-soluble antioxidants with these oxidants. Among the lipid soluble antioxidants in plasma and LDL, ubiquinol-10 is consumed before α-tocopherol and appears to protect the lipids more effectively (Frei and Ames,1993).

Oxidative damage has occurred in association with the release of free iron, copper, haem or haemoglogin into plasma and /or tissues. This can occur following iron or copper overload or following haemorrhage and haemolysis. Under these conditions the metal binding proteins of plasma become of primary importance in preventing peroxidative damage: transferrin, lactoferrin, caeruloplasmin, albumin, haemopexin, and haptoglobin. The low molecular weight antioxidants, including α-tocopherol, are of secondary importance, with the only possible exception of uric acid, which can chelate iron (Frei and Ames, 1993).

ANTIOXIDANTS IN CEREBROSPINAL FLUID

One tissue that is very prone to increased free radical reaction following damage is the brain. Unlike plasma and normal synovial fluid, cerebrospinal fluid has very little in the way of metal-binding capacity, i.e. it lacks the metallic ion binding proteins, so that iron complexes released into cerebrospinal fluid from damaged brain (e.g following haemorrhage) tend to stay there and are available to promote radical reactions. Damaged brain undergoes

lipid peroxidation at a fast rate (Halliwell,1987).

VARIABLES WHICH WILL INFLUENCE OXIDATIVE DAMAGE

Iron

Iron is transported around the body attached to the protein transferrin. It is stored in the protein, ferritin. Iron is unloaded from transferrin within the cell, the excess goes into ferritin and the rest into an iron pool believed to consist of low molecular weight iron chelates with iron being attached to ligands such as ATP, ADP or citrate and possibly attached loosely to various proteins and to cell membranes. These chelated forms of iron can promote $OH^{\bullet}$ formation (Halliwell,1987).

Injections of iron into piglets and rats can exacerbate the effects of Se/vitamin E deficiency (Dougherty *et al*,1981). Fe, being a redox catalyst, both multiplies the number of radicals produced and creates more reactive and damaging species, unless it is strictly compartmentalised and bound to a restrictive chelator (Golden and Ramdath,1987).

Generally, the cell is adequately protected against the damaging effects of Fe^{2+} by effective complexing of the iron (as in transferrin, haemosiderin, ferritin, etc.) and by compartmenting these 'pools' of Fe away from susceptible membrane sites. If the cell structure is damaged such that Fe is 'decompartmentalised' or so that excess Fe can gain access to intracellular organelles then extensive and rapid peroxidation may ensue (Slater, 1987).

At normal physiological pH, transferrin will not release Fe. However, at acidic pH, transferrin does release its Fe (as it has evolved to do within the cell). The value usually quoted for the pH at which transferrin releases Fe is around 5.5 (Halliwell, 1987).

Because of its high reactivity and short half-life, $OH^{\bullet}$ will not get very far from its site of formation. Hence what really determines the nature of the damage done by $O_2^{\bullet-}$ and H_2O_2 is the location within the organism of metal ion complexes that will promote $OH^{\bullet}$ formation. For example, if there are metals bound to DNA then DNA fragmentation will result, but if the metals are stuck to membrane lipids there will be lipid peroxidation (Halliwell,1987).

Iron chelating agents have been used to protect against oxidative damage. The compound, desferrioxamine has been shown to be particularly effective (Halliwell,1987).

Copper

Injections of copper into rats can exacerbate the effects of Se/vitamin E deficiency (Dougherty *et al*,1981). Dietary deficiency of copper will decrease tissue SOD activity, since copper is a functional component of SOD.

Iron and copper are needed in key protective enzymes such as transferrin, catalase (Fe) and Cu/Zn superoxide dismutase (Cu) however excessive supplementation with copper or iron will saturate all potential binding sites and raise the levels of these metals in their free states which may then catalyse oxidative reactions. This is evidenced in the spectacular sudden death syndromes associated with acute and chronic copper toxicity.

Manganese

Since Mn is a functional component of SOD, dietary deficiency will decrease tissue SOD activity (Arthur,1982).

Sulphur containing amino acids

Deficiencies of vitamin E and/or selenium result in impaired metabolism of the sulphur containing amino acids. The dietary content of S-amino acids can modify the effects of Se/vitamin E deficiency in chickens and rats. Additional methionine to vitamin E/Se deficient diets in rats will prevent oxidation induced liver necrosis (Combs *et al*,1980).

Environmental temperature and feed intake

Experiments in rats fed vitamin E/selenium deficient diets demonstrated that low environmental temperature or restricted feed intake reduced oxidative damage to the liver (Naftalin,1954).

Physiological exertion

In animals it appears that vitamin E deficiency myopathy may be precipitated by exercise, and that vitamin E deficient animals have substantially reduced exercise endurance. Vitamin E deficiency may exacerbate exercise-induced damage (Jackson, 1987).

Polyunsaturated fatty acids

Fatty acids are straight-chain hydrocarbon carboxylic acids, which can be either saturated or unsaturated depending on the presence of carbon-carbon double bonds in their structure. Saturated fatty acids have no double bonds whereas unsaturated types have one (mono-unsaturated) or more (poly-unsaturated). Due to the lengthy chemical nomenclature of the fatty acids, a shorthand notation has been devised. The fatty acids are described by their number of carbon atoms, number of double bonds, and the position of the first double bond as numbered from the methyl end of the molecule. e.g.linoleic acid can be designated as 18:2n-6, which describes an 18 carbon acid having 2 double bonds with its first double bond located between carbon numbers 6 and 7 as counted from the methyl end. The location of this double bond characterizes it as a member of the n-6 (or omega 6) series of fatty acids.

There are four families of unsaturated fatty acids in mammals. These have either palmioleic, oleic, linoleic or linolenic building blocks. The two essential in mammals, are those based on linoleic (n-6) and linolenic acids (n-3) and dietary deficiency can result in a variety of diseases. When present at high concentrations in animal tissue the vitamin E requirement goes up. Neither linoleic nor linolenic acid can be synthesized in mammalian tissues but both can undergo further desaturation and chain elongation to give two series of derivatives, the omega 6 and omega 3 series. Competitive inhibition occurs between these two series and the balance is determined by the ratio of linoleic:linolenic acid in the diet (Sanders,1988).

Polyunsaturated fatty acids have two main functions in the body:-

i. as the primary constituent of subcellular membranes

ii. as precursors of eicosanoids

Singer and Nicholson (1972) proposed that membranes were composed of inner and outer bilayers of phospholipids and integral proteins and that these were mobile as a result of the fluidity of the membrane. The fluidity of the membrane, which is extremely variable, is dictated mainly by the polyunsaturated fatty acid content of the phospholipids. High levels of PUFA and low levels of cholesterol tend to increase membrane fluidity.

Many polyunsaturated fatty acids can act as substrates for eicosanoids (prostaglandins, thromboxanes, prostacyclins, leukotrinenes) with the most commonly occurring and biologically active prostanoids arising from arachidonic acid. These compounds are formed when arachidonic acid (or other PUFA) is released from membranes by phospholipase and converted by enzymatic action to an endoperoxide to form the eicosanoids. Eicosanoids are mediators of inflammation, in particular they cause leucocyte migration and oedema. The inhibition of their formation is the mode of action of most non-steroidal anti-inflammatory drugs. The eicosanoids, prostaglandins are a group of closely related 20-carbon unsaturated fatty acids which exert profound effects on a wide variety of tissues.

When the membranes of platelets, white blood cells, and tissue cells become activated due to some chemical or physical insult, n-6 series fatty acids (primarily arachidonic) are metabolically transformed into prostaglandins of the 2 series and leukotrienes of the 4 series. These prostaglandins are believed to play a major role in inflammation. When n-3 series fatty acids are present, however, series 3 prostaglandins and series 5 leukotrienes may be formed which are less inflammatory (Bauer,1994).

The formation of these peroxides is an essential process which is continuously ongoing in all body cells and it is essential that cell membranes are protected from the resultant free radicals. This appears to be the principal function of vitamin E.

In diseases caused by oxidative damage, we are concerned with the inherent instability of unsaturated fatty acids. The chemical structure of these acids makes them particularly susceptible to degradation by peroxidative mechanisms. Peroxidisability refers to the relative rates at which unsaturated fatty acids form peroxides. The peroxidisability ratios for oleic (a monounsaturated fatty acid), linoleic and linolenic acids are 1:12:25 respectively (Stirling,1965). Chain length and degree of unsaturation influence the rate of oxidation of polyunsaturated fats. The rate of oxidation decreases with increasing chain length (Sanders,1988).

In vivo oxidant protection by the dietary incorporation of the monounsaturated fatty acid, oleic acid, has been demonstrated in mice challenged with the toxin, paraquat. Paraquat causes severe oxidative damage through the generation of the superoxide anion radical. This toxicity is exacerbated by vitamin E or selenium deficiency. It is theorised that the reduction in polyunsaturated fatty acids by replacing with the monounsaturated fatty acid decreases the susceptibility of membranes to oxidative attack (Fritz *et al*,1994).

Grass and pasture plant lipids contain between 60 and 70% linolenic acid. The rumen is a potent lipid hydrogenator, saturating the dietary unsaturated fatty acids (Noble *et al* 1974, Wichtel *et al* 1996). In spite of this it is possible for the lipids to pass through the rumen unsaturated under certain circumstances. Calves turned out to spring pasture can allow plant lipids through the rumen without hydrogenation, demonstrated by an increase in plasma linolenic acid of 230% (McMurray, 1980). It is also possible to protect unsaturated fats by coating them. The first process to protect dietary fats from ruminal metabolism was developed in Australia by Scott *et al* (1970); vegetable oils were emulsified with sodium caseinate and spray-dried to produce fine particles which were then treated with formaldehyde to form a cross-linked matrix that protected the protein and entrapped oil from attack by rumen microbes. The formaldehyde-treated protein lipid supplements pass largely undigested from the rumen into the abomasum, where the acidic conditions weaken the aldehyde-protein link and allow digestion and absorption of both lipid and protein. Considerable research is currently being undertaken in this field to manipulate the fat composition of meat and milk (Scott and Ashes,1993).

THE PUFA CONCENTRATION OF TISSUES AND MEMBRANES

PUFA concentration can vary between tissues, and within cells certain organelle membranes have particularly high concentrations of PUFA e.g. mitochondria and endoplasmic reticulum. The PUFA concentration of subcellular membrane phospholipids varies greatly among species, tissues within species, membranes within cells and even among different sites in the membrane (Lee 1985).

The likelihood of auto-oxidation occurring in a particular membrane will be determined principally by the degree of unsaturation of the fatty acids in the membrane, secondly by the rate of production of peroxides from the PUFA and thirdly by the concentration of antioxidants including vitamin E within or at the surface of the membrane.

Kennedy and Rice demonstrated selective pathological changes in the cardiac conduction systems in calves deficient in vitamin E and selenium (Kennedy and Rice 1988). They hypothesised that these cells may be more susceptible than contractile cardiocytes because Purkinje cells may have more highly unsaturated membrane phospholipid and lower concentrations of lipoperoxidation antagonists, such as vitamin E, glutathione peroxidase, superoxide dismutase, catalase, vitamin C, and vitamin A than contractile cardiocytes. Similar pathological lesions have been found in humans with Keshen disease, a selenium-responsive cardiomyopathy of humans in China. Selective degeneration of the cardiac conduction system has been reported in children with kwashiorkor (Sims, 1972). Selenium deficiency is a feature of this disease (Burk *et al*, 1967).

Dietary modification of PUFA in membranes

The PUFA concentration of subcellular membranes is very susceptible to dietary manipulation, as demonstrated in cattle fed protected corn oil (high in linoleic acid) or linseed oil (high in linolenic acid) (Rice and McMurray 1982). In both instances the predominant fatty acid of the dietary oil is incorporated into tissues. In the case of protected linseed oil the linoleic acid is incorporated very rapidly into tissue phospholipids (Rice and Kennedy 1988). This means that feeding high levels of unsaturated fats to livestock will increase the PUFA

concentration of membranes and will therefore increase their susceptibility to peroxidative damage.

Changes in the balance between omega 6 and omega 3 fatty acids may affect the physical properties of the membrane as well as the activity of membrane bound enzymes (Sanders,1988). Theoretically dietary n-3 acids could modify the composition of membrane phospholipids in practically all cells of the body and could be useful in the prevention or treatment of thromboembolic, autoimmune, inflammatory, and possibly neoplastic diseases but as yet this has not been confirmed (Bauer,1994). Currently there is considerable interest in altering the balance between omega 6 and omega 3 fatty acids in the therapy of certain inflammatory diseases of animals and man (Alexander *et al*,1987; Stitt and Johnson,1990; Sinclair,1991; Skjervold 1992, Horrobin,1992; Harvey,1993 and Bauer,1994).

Unsaturated fats are hydrogenated (saturated) in the rumen (Noble *et al* 1974) thus protecting the animal from absorption of unsaturated fats. The hydrogenation is not complete and some unsaturated fatty acids are absorbed. It would appear that this amount is relatively small, as the depot fat of ruminants contains only 1-3% of unsaturated fatty acids (Jenkins *et al*,1969). This hydrogenation does not occur in monogastric animals and in preruminants and presumably would not occur in rumen malfunction. Normally the fatty acid composition of bovine fat is apparently unaffected by the nature of dietary fat (Shorland,1950), however Cabezas *et al* (1965) reported that beef cattle diets containing high levels of starch increased the level of unsaturated fat. Increased unsaturation has been associated with more marbling of meat (Skelley *et al*, 1973) and conversely a high degree of saturation is associated with decreasing amounts of marbling (Cook *et al*,1965).

Shaw *et al (*1960) reported a higher degree of unsaturation in kidney fat when flaked corn was fed with ground alfafa. This must have been associated with greatly altered rumen fermentation as indicated by changes in rumen molar proportions of acetate and propionate. Tove and Matron (1962) found morphologially different rumen flora in lambs that were fed diets high in soluble carbohydrates which produced a significant increase in unsaturation of subcutaneous adipose tissue. Their results suggested a reduction of the hydrogenation capacity of rumen microorganisms due to either a lowered activity of fatty acid hydrogenation enzymes, a reduced concentration of hydrogen donor compounds or production of an inhibitor of the enzyme system. Cabezas *et al* (1965), demonstrated a high inverse relationship between ruminal acetate and dietary starch and a high positive relationship between ruminal propionate and dietary starch.

Henriques and Hansen (1901) and Hilditch (1947) (as quoted by Cook *et al, 1965*) postulated that the degree of unsaturation of a fat was dependent on the temperature of the specific area from which the fat was obtained. Cramer *et al* 1961 (as quoted by Cook *et al*, 1965) showed a significant correlation between the temperature of an area and the level of unsaturation of the lipid. Unsaturated fats have a lower melting point than saturated fats and therefore would be more suited to cold areas of the body i.e. they would not easily solidify in the cold.

Rice and Kennedy (1988) have proposed that the site of oxidative damage can depend on deficiency of essential nutrients as well as a number of additional variables within the membrane where peroxidosis is occurring. These include :-

1. the inherent degree of unsaturation of the PUFA within the membrane
2. the degree of unsaturation of the PUFA within the membrane resulting from a high dietary input of PUFA and/or factors leading to increased membrane PUFA desaturation
3. the intensity of the initiation of the tissue peroxidosis whether chemical or enzyme related
4. the inherent capacity of the tissue or membrane and its environs to store peroxidation antagonists, including vitamin E, glutathione peroxidase, catalase, β-carotene, superoxide dismutase etc.
5. the levels of such antagonists in the membrane as a result of dietary input and their bioavailability
6. the importance and function of the damaged membrane in relation to its parent cell and to other cells which depend on products elaborated in the parent cell

Consideration of these variables indicates that it is not surprising that oxidative damage deficiency can result in so many different disease manifestations.

DETRIMENTAL EFFECTS OF TISSUE PEROXIDES

Uncontrolled lipid peroxidation results in a variety of detrimental effects (Rice and Kennedy, 1988)**.** There is a decrease in the concentration of phospholipid PUFA and an increase in cross-linking of peroxidised PUFA. Both of these decrease membrane fluidity, resulting in increased membrane fragility, increased permeability to a variety of substances and abnormalities in protein function as a consequence of protein denaturation. The peroxides could act as precursors for prostanoid synthesis, leading to further alterations in prostaglandin/ prostacyclin/ thromboxane balance with potentially detrimental effects on cell metabolism. The free radicals may also damage nucleic acids leading to somatic mutation and scrambling of genetic information with a consequent risk of carcinogenesis.

BIOCHEMICAL MARKERS FOR PEROXIDATION

Demonstration of increased free radical activity in biological tissues is a relatively difficult process since no single test is currently accepted as ideal for the purpose. Oxidation can damage many biological molecules. Protein and DNA can be significant targets with lipid peroxidation occurring late in the process. The breakdown of lipid peroxides by free radical damage results in the production of thiobarbituric acid (TBA)-reactive substances such as malonaldehyde. Halliwell and Chiraco (1993) describe an HPLC based TBA test. Degradation products of the lipid peroxide will also produce a series of fluorescent compounds. These are formed in combination with amino groups, e.g. from amino acids or proteins, and could therefore be used for evidence of peroxidation. Under certain circumstances, peroxidation will lead to the production of hydrocarbon gases such as ethane and pentane. Production of these gases *in vivo* can be detected in exhaled breath. Such gases have only been consistently detected when peroxidation occurred in either liver or lung. In degenerative myopathy the levels of muscle enzymes such as creatinine phosphokinase rise in the serum (McMurray *et al*, 1983).

DISEASES KNOWN TO BE CAUSED BY NUTRITION RELATED OXIDATIVE DAMAGE

The two most common nutritional deficiencies which result in oxidative damage are deficiencies of selenium (a component of glutathione peroxidase) and vitamin E. It is convenient to consider the clinical signs observed in vitamin E /selenium deficient animals according to the organ system most affected. These disorders are of 5 general types.

i. **Vascular disorders** - clinical signs being due primarily to capillary leakage or loss of erythrocyte integrity

ii. **Nutritional myopathies** - clinical signs involving either degeneration of muscle fibres of either the striated, smooth, or cardiac types.

iii. **Encephalopathies** - clinical signs involving the brain.

iv. **Vital organ pathologies** - clinical signs involving the liver, kidney, or pancreas.

v. **Reproductive disorders** - Clinical signs involving spermatogenesis, sperm or ovarian functions, or foetal development.

These categories are not mutually exclusive, as it is likely that several specific organ pathologies actually may be caused by microvascular disorders. Vascular lesions have been reported in the brains of vitamin E deficient chicks with encephalomalacia, in the subcutis and skeletal muscles of Se/vitamin E deficient chicks with exudative diathesis and vascular damage has been shown to cause the testicular necrosis in rats with cadmium toxicity which can be prevented with selenium supplementation (Van Vleet *et al*,1977)

Some vitamin E /selenium deficiency can be exacerbated by feeding diets containing large amounts of PUFAs

Vitamin E and selenium deficiencies of domestic animals have been reviewed by Lannek and Lindberg

(1975). Symptoms of vitamin deficiency are not always observed. One of the best indicators is the weight gain of test animals.

Degenerative diseases and aging

Free radicals are believed to influence the aging process (Floyd,1991). It has been postulated that the normal level of antioxidant defences is not fully efficient, so that a fraction of reactive oxygen species (ROS) escape elimination. These ROS inflict molecular damage, some of which is irreparable and accumulates with age, thereby causing functional attrition associated with degenerative disease and aging. Some data is now available to support this hypothesis. Studies in the fruit-fly, *Drosophila melanogaster,* have shown that by genetically engineering the flies to carry three genes (instead of one) for the expression of copper-zinc superoxide dismutase and catalase, the life-span of the flies was extended by one-third, there was a lower amount of protein oxidative damage, and a delayed loss in physical performance. The results of this study underscore the importance of an optimal balance between SOD and catalase. In previous experiments, the overexpression of Cu-Zn SOD alone or catalase alone had only a minor incremental effect (Orr and Sohal,1994).

Table 1: Examples of vitamin E and selenium deficiency diseases, demonstrating the wide variety of tissues affected by deficiency and also the many species of animals affected.
(Adapted from Rice and Kennedy, 1988, and Scott,1980 and El Boushy,1988).

Tissue	Species	Diseases
Vascular system (embryo)	Rat, hamster, mouse, hen, turkey	Embryonic degeneration
	Chick, turkey	
Vascular system		Exudative diathesis
	Pig	Enlarged hocks
		Mulberry heart disease
	Chicken	
Reproductive system	Cattle, sheep	Lowered hatchability
		Embryonic degeneration,
	Rat, Guinea pig, hamster, dog, cock, rabbit, monkey	Retained placenta
		Male sterility
	Chicken	
Digestive system		Gizzard erosion
	Rat, pig, bovine, fish, chicken	
Liver		Necrosis, telangiectasis
		Lipoid degeneration
	Chick, mouse	
Pancreas	Man, chick, rat.	Fibrosis
Erythrocytes	Chick	Haemolysis
Cerebellum	Rat, mouse, mink	Encephalomalacia
Kidney		Degeneration of tubular epithelium
	Mink, pig, chick	
		Steatitis, "Yellow fat disease" *
Adipose tissue		
	Rabbit, guinea pig, monkey, mouse, duck, mink, lamb, calf,	Skeletal and cardiac myopathies.
Myopathies	chicken, turkey.	

* Yellow fat disease

In 1945, Dam and Granados showed the presence of peroxides in adipose tissue of rats and chicks fed diets deficient in vitamin E and rich in polyunsaturated fatty acids. This condition was characterised by a yellow-brown discoloration of the fat tissue. Later it was found that steatitis or yellow fat disease due to vitamin E deficiency also occurred in vitamin E deficient mink and pigs (Scott, 1980). Is it possible that this could occur in cattle under certain dietary conditions?

DISEASES INVOLVING OXIDATIVE DAMAGE TO BLOOD VESSELS

Oxidative damage is believed to be involved in vascular damage in human atherosclerosis, thrombophlebitis, intermittent lameness and kwashiorkor (Golden and Ramdath, 1987), mulberry heart disease of pigs and exudative diathesis of chickens. McMurray *et al (*1983) observed extensive vascular lesions in some cattle from a group in which they were experimentally inducing white muscle disease. Hydrogen peroxide, a product of activated neutrophils, has been shown to produce irreversible endothelial cell injury and death (Bradley *et al, 1993). Oxidative damage has been associated with microvascular permeability and morphology in the equine jejunum*

(Dabareiner et al,1995).

Human atherosclerosis

Low density lipoproteins (LDL's) lodge in the arterial wall and become oxidised damaging nearby tissues (Ross,1993). A protective role by vitamin E has been demonstrated in epidemiologal studies (Stampfer *et al* 1993, and Rimm *et al* 1993). Although studies in humans and animals indicate that a higher intake of polyunsaturated fats in relation to saturated fats is beneficial to cardiovascular health, some findings suggest that a diet high in polyunsaturated fats that are insufficiently protected by antioxidants, such as vitamin E, may carry a higher risk of atherosclerosis (Kubow, 1993).

It is believed that the initial lesion in the sequence of events leading to the formation of atheromatous plaque is damage to the arterial endothelium. *In vitro* experiments with cell cultures of porcine pulmonary artery endothelial cells have demonstrated that they can be irreversibly damaged by linoleic acid hydroperoxides and that such damage is inhibited by previous incubation of the cells with vitamin E. *In vivo* experiments in rabbits demonstrated damage and denudation of aortic endothelial cells when lipid hydroperoxide was injected into the bloodstream (Duthie *et al*,1989).

Currently there is much interest in the prophylactic effect of polyunsaturated fats on cardiovascular disease, particularly in relation to omega-3 fatty acids in fish oils. However, the relative ease of fatty acid peroxidation both by endogenous free radical activity and through oxidation in the gastrointestinal tract may lead to the formation of lipid hydroperoxides which will be detrimental if antioxidant levels are low (Duthie *et al*,1989).

Thrombophlebitis and intermittent lameness of humans

Several human diseases have been shown to be alleviated by dietary intake of vitamin E. Among these are thrombophlebitis and intermittent claudication (lameness), both of which involve blood flow, particularly in the extremities of elderly people. Controlled studies by Haeger in Sweden showed that daily administration of 300-600 mg of vitamin E to half of a group of 227 elderly men caused a marked improvement in blood circulation in the extremities (Haeger,1968,1974).

Kwashiorkor and other diet-related oedemas in humans

Golden and Ramdath (1987) proposed that kwashiorkor results from an imbalance between the production of free radicals and their safe disposal. The disease is characterised by oedema, fatty liver, pigment changes, diarrhoea, immunoincompetence and mental changes and is associated with low concentrations of vitamins E and A, β-carotene, zinc, manganese and selenium in conjunction with high plasma ferritin levels. Iron can act as a catalyst in oxidative damage and it is postulated that the high ferritin levels in affected children may predispose them to the condition. Golden and Ramdath showed that children with unequivocal kwashiorkor syndrome had very low levels of glutathione and concluded that this was because of increased consumption rather than decreased production.

Oedema has been attributed to polyunsaturated fats in the diet in two human conditions. Oedema can occur in vitamin E deficient premature infants stressed by a diet high in polyunsaturated fats. "Epidemics" of oedema in India during the last century which were clinically similar to kwashiorkor, were caused by contaminated cooking oil with this presumably producing an oxidative stress (Golden and Ramdath,1987). Deaths associated with contaminated cooking oils have been attributed to aflatoxins which are oxidants.

Exudative diathesis in the chick

This is characterised by generalized oedema of the subcutaneous fatty tissues and muscle, associated with an abnormal permeability of the capillary walls and often large accumulation of fluid under the skin. The condition occurs on certain types of vitamin E deficient diets and is responsive to vitamin E (Scott, 1980).

Mulberry heart disease in the pig

This disease occurs mainly in pigs 2-4 months of age but has been observed in animals from 3 weeks to 4 years of age. The disease can be readily reproduced by raising piglets on a diet deficient in selenium and vitamin E. It is believed that it occurs when there are high requirements for vitamin E such as diets containing large amounts of unsaturated fats. Myocardial degeneration occurs and microangiopathy affecting arterioles in many organs. The changes are seen in the heart, kidneys, liver, stomach, intestine, mesentery, skeletal muscle, and skin. There appears to be gradation in the severity of change, from endothelial swelling with increased permeability to necrosis of smooth muscle cells in the media. The basic appearance includes fibrinoid necrosis of the arteriolar walls, formation of hyaline thrombi, disruption of endothelium and necrosis of smooth muscle cells (Jubb *et al,* 1993).

Nutritional Myopathy (White muscle disease) in various species

White muscle disease was initially reported as a disease in young suckling or bucket fed calves, lambs and foals and caused by a deficiency in vitamin E and/or selenium. It has since been identified in foetuses and newborn animals from vitamin E and/or selenium deficient dams, and in ruminant cattle in the age group 6 to 24 months, generally following turnout to pasture in the spring after grain feeding in the winter (Anderson *et al,* 1976). Allen *et al* (1975), showed that the winter diets consumed by affected yearling cattle were deficient in vitamin E and selenium. A Se-vitamin E deficiency associated myopathy has been reported in adult horses in Canada (Owen Rap *et al,*1977; Van Vleet,1980). As well as white muscle disease, other rarer forms of nutritional myopathy have been recognized. One form occurs in bovines and is and termed "Flying scapula" There is severe, bilateral atrophy of the serratus ventralis muscle causing a distinctive clinical appearance of scapulae raised dorsally. This condition is believed to be responsive to vitamin E and selenium (Hannam *et al,* 1994).

In Australia, white muscle disease most commonly occurs in areas where the soil and pasture are deficient in selenium and frequently is initiated by lush green growth. White muscle disease has also been reported in Australia in sheep grazing wheat stubble and being fed grain. This condition has been attributed to low vitamin E levels (Steele *et al,* 1980; Peet *et al,* 1980).

There are two major syndromes of nutritional muscular dystrophy: an acute form of myocardial dystrophy, which occurs most commonly in young calves and lambs and occasionally foals - and a subacute form of skeletal muscular dystrophy, which occurs in older calves and yearling cattle affecting animals up to two years of age on turnout to Spring pasture; weaned calves are affected within one week and suckling calves within three weeks of turnout (McMurray and Rice,1982). The two forms are not mutually exclusive (Blood and Radostits, 1989).

The mechanism of the aetiology and pathogenesis of nutritional degenerative myopathy in young cattle remained obscure mainly because many cattle which are deficient in vitamin E and selenium do not succumb to the disease. A deficiency of these nutrients has therefore been considered as a predisposing factor rather that the direct initiator of the disease (Rice and McMurray,1986). Dietary polyunsaturated fatty acids, bad weather and unaccustomed exercise have been suggested as initiators. Since the 1950s it has been known that diets high in PUFAs can induce myopathy (Rochester and Caravaggi,1971). However experimental work by Rice and Murray (1986) suggest that when tissues of cattle are sufficiently depleted of vitamin E and selenium, spontaneous myopathy will ensue. It is most likely that exogenous modulators such as polyunsaturated fatty acids from grass will act by increasing the susceptibility of muscle membranes to peroxidosis.

McMurray *et al (*1983) demonstrated dramatic changes in both absolute and relative fatty acid composition in plasma when winter fed calves were turned out to pasture. A major increase also occurred in plasma α-tocopherol. While the linoleic acid content decreased as a proportion of the total, linolenic acid increased by up to twenty-fold, both in absolute terms and as a proportion of the total fatty acids concentration of plasma. The change occurred in the first 5 to 6 days after turnout, after which it plateaued (McMurray *et al,*1983).

Tissue analysis indicates that a substantial increase in the content of PUFA in muscle is not essential for the development of myopathy. Exogenous PUFA in the diet may be oxidised before reaching cell membranes producing hydroperoxides which will initiate lipid peroxidation in the membranes (Arthur,1982).

McMurray *et al* (1983) were able to reproduce white muscle disease experimentally by feeding 4-10 month old calves, which were deficient in selenium and vitamin E, protected linseed oil which had had its vitamin E removed. Linseed oil was used for these experiments because its fatty acid composition closely resembles that of grass (high linolenic, low linoleic acid). The mean plasma α-tocopherol of the depleted calves was 6.72 μmol/L and fell precipitously to 0.29 μmol/L with the feeding of linseed oil. This experimental procedure induced nutritional muscular dystrophy and serum creatinine phosphokinase (CPK) levels rose within 2-3 days. Mean plasma linolenic acid of the PUFA-fed calves rose from 2% of the total fatty acids to 24.6% after 10 days of PUFA feeding. Within 6 to 11 days of feeding the protected linseed oil, clinical signs, biochemical changes, and pathological changes similar to those reported in natural outbreaks of nutritional myopathy, occurred (Kennedy *et al*,1987).

The results of adding PUFA to the diet confirm that if dietary linolenic acid is protected from ruminal hydrogenation and fed to E-Se depleted calves, it is rapidly absorbed into the blood stream and is associated with a concomitant increase in plasma CPK activity as a result of myodegeneration.

Experiments by Arthur (1988) to induce white muscle disease in calves suggest that other factors such as unaccustomed exercise, may be necessary to induce the clinical disease.

Nutritional myopathy in ruminants responds rapidly to the administration of Se or vitamin E except when it is induced by high dietery concentrations of polyunsaturated fatty acids (PUFA) when only vitamin E is effective (Arthur, 1982)

There is variation in the susceptibility of different muscle fibre types. In fast twitch muscle, where the ATP required for muscle contraction is derived from glycolysis, there is a low oxygen requirement. Slow twitch fibres, which depend on oxidative respiration for ATP synthesis, have a much higher oxygen demand and hence the possibility of increasing the production of toxic oxygen metabolites and the severity of peroxidation (McMurray *et al* 1983). Similarly lesions of white muscle disease are most severe in the left ventricle because of its greater work load and metabolic activity. In cases of congenital white muscle disease the right ventricle is predominantly affected because of its higher workload in foetal physiology.

In degenerative myopathy there is disruption of the sarcolemma, the membrane system surrounding the muscle fibre, resulting in leakage of the soluble cellular contents which can be monitored by measuring the muscle enzymes, CPK and lactate dehydrogenase (LDH). McMurray *et al (*1983) observed extensive vascular lesions in some cattle from a group in which they were experimentally inducing white muscle disease. The pathology of one animal was similar in some respects to that seen with mulberry heart disease (dietetic micro-angiopathy) in the pig. In this animal there was proliferation of the capillary endothelium leading to occlusion of some blood vessels. The site of these lesions was not mentioned. In another animal, damage to blood vessels as a result of fibroid necrosis, was widespread. This animal showed post-mortem changes similar to those seen in exudative diathesis in the chicken. Vascular lesions have not been reported in naturally occurring cases of white muscle disease.

The ultrastructural alterations to blood vessels in nutritional cardiomyopathy of selenium-vitamin E deficient swine have been described by Van Vleet *et al* (1977). Light and electron microscope study revealed myocardial arteriolar damage characterized by segmental fibrinoid accumulation in vessel walls and by scattered fibrin thrombi. Ultrastructural study disclosed extensive subendothelial and inner wall accumulations of dense granular deposits of serum proteins and masses of fibrin in arterioles. Endothelial cells of these arterioles were loosely attached to each other. In arterioles with fibrin thrombi, the endothelium was disrupted. In mildly injured arterioles, increased endothelial permeability resulted in leakage of blood proteins into the vessel wall to produce accumulation of fibrinoid. In severely injured vessels, endothelial integrity was destroyed, smooth muscle cells were necrotic and thrombosis had developed. Initiation of these arteriolar lesions was apparently the result of lipoperoxidative damage to endothelial cell membranes that lacked protection by selenium-vitamin E.

The histopathologic and ultrastructural alterations in the myocardium of calves with experimentally induced white muscle disease have been described by Kennedy and Rice (1992). Myocardial vascular alterations were seen in four of five calves with severe histopathologic myocardial alterations. Many capillaries had swollen endothelial cells that frequently resulted in luminal occlusion. Thickening of the tunica media was a common alteration in large arterioles. Hyaline material and pyknotic nuclei were occasionally seen in the media of such

arterioles. Arteriolar alterations were associated with infiltration of the adventitia with mononuclear cells.

Their experiments posed one important question. How can animals become diseased when exposed to a myopathic challenge and not be protected by the vitamin E which is entering the blood stream at the same time as the linolenic acid? In previous experiments vitamin E was shown to be protective when administered before turnout. This may be explained by the slow uptake of α-tocopherol following oral supplementation compared with the rapid uptake of PUFA by cells. Experiments by Arnold *et al*, 1993 demonstrated that the accumulation of vitamin E in the *longissimus lumborum* muscle required 12 to 18 weeks before the levels equilibrated with dietary intake.

DISCUSSION

Free radicals can arise from exogenous sources e.g. radiation, toxins, bacterial and viral infections, and ingestion of polyunsaturated fats, and from endogenous sources in normal cell metabolism and in inflammation.

A diverse array of antioxidants is available in the body to provide lines of defence against the harmful effects of free radicals. The two most important nutritional deficiencies which result in a deficit of antioxidants are deficiencies of selenium (a component of glutathione peroxidase) and vitamin E. With vitamin E in particular many dietary, physiological and pathological factors can influence its uptake and utilization. The feed used in intensive livestock industries is stored for varying lengths of time and hence almost invariably depleted of natural vitamin E. Supplementation is essential, and must be given in an effective form and at a rate which will maintain adequate blood and tissue levels.

The amount of polyunsaturated fatty acids (PUFAs) absorbed from the diet is an important factor in initiating disease. Not only can the PUFAs produce free radicals, they are also readily taken up by membranes, and can thus alter the chemical make-up of membranes and make them more susceptible to oxidative damage. PUFAs are absorbed readily in monogastric animals but in ruminants PUFAs are hydrogenated (saturated) by the ruminal microflora. This may in part explain why pre-ruminant calves and lambs are more susceptible to oxidative damage than the older animals with fully functional rumens. From studies in meat science, it would appear that ruminants on a high starch diet are not able to completely hydrogenate PUFAs and will deposit fat with a higher than normal PUFA content. It would be logical to conclude that such animals would be more susceptible to oxidative damage. This should be an important consideration in the diagnosis and prevention of disease in feedlot cattle which are on a high starch diet. Similarly current research into the alteration of fatty acid composition of beef and milk must take into account the increased risk of nutrition related oxidative damage both in the production animal and the human consumer.

One of the most significant "experiments" in human nutrition has occurred over the last few decades with the increased consumption of polyunsaturated fats by Western populations. Without a concomittent rise in antioxidant levels, it could be surmised that this increased consumption would result in increased free radical production and hence oxidative damage. The impact of this dietary change on oxidative damage and eicosanoid production has yet to be realized.

The susceptibility of cell membranes to oxidative damage depends on many variables: the chemical nature of the membrane, its degree of metabolic activity, and the balance between oxidants (free radicals) and antioxidants. The chemical nature of membranes depends on the function of the membrane, the absorption of dietary factors such as PUFAs, the type of PUFAs, and the location of the membrane in the body e.g. in relation to the temperature of the body in that region. It has been shown that peripheral fat is higher in unsaturated fats than core fat and would therefore be more susceptible to oxidation. It could be postulated that the extremities would be more susceptible to damage because of their higher content of PUFAs. The disease manifestations of oxidative damage are therefore diverse and even within one species various body systems can be affected, with animals in different age groups or on different diets displaying dissimilar signs of disease.

Blood vessel damage has been related to oxidative damage in a number of species, and can occur in conjunction with oxidative damage to other tissues such as muscle and brain. It is likely that blood vessels are particularly susceptible to exogenous free radicals in the circulation or to endogenous damage at times of high metabolic activity.

APPENDIX 8

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Nornews Rural Magazine April 1997

Study finds Vitamin E can reduce heat stress

The lack of vitamin E has been linked to hind leg swelling and heat stress cattle deaths in feedlots by a NSW Agriculture researcher.

In 1990 NSW Agriculture's Special Veterinary Research Officer at Armidale, Dr Barbara Vanselow, began investigating an unexplained condition of leg swelling feedlot cattle.

Working at the Armidale Veterinary Laboratory, as part of the Cooperative Research Centre for the Cattle and beef Industry with funding from the Meat Research Corporation, Dr Vanselow's research has included feedlots in northern NSW and southern Queensland.

The results show that a lack of vitamin E in feedlot cattle is responsible for damage to small blood vessels in the limbs, particularly in the lower hind legs.

It appears that this damage impairs the body's temperature regulation and results in an inability of the animal to cope with heat stress.

The condition can also occur when fattening cattle for show and when drought feeding.

The condition is first seen as lameness, fluid swelling of the hock joint, progressing to generalised swelling down to the hoofs and sometimes up to the groin.

The skin directly above the hoof and around the pastern is reddened, swollen and tight. Affected animals were inclined to have elevated temperatures, she said.

"When affected tissue is seen under a microscope there is damage to cells lining small blood vessels which allows leakage of fluid and protein into surrounding tissue.

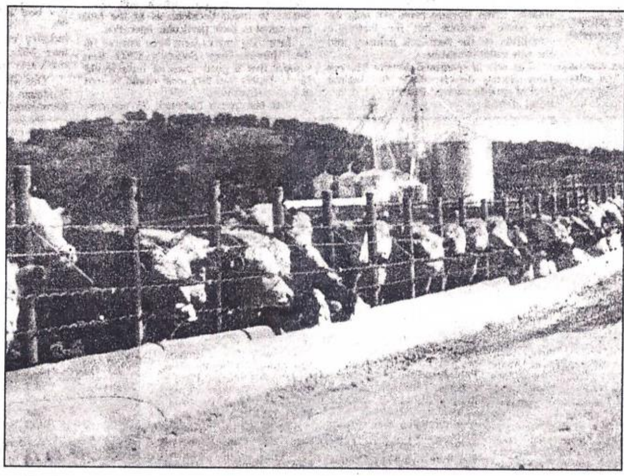
"Superficial blood vessels and those around the joints are most severely affected leading to inflammation, haemorrhage and swelling.

"Comprehensive investigations constantly showed the condition occurred during prolonged hot weather in animals fed low levels of supplementary vitamin E. These same animals had low blood levels of vitamin E.

"Blood levels of vitamin E dropped as the number of days on feed increased.

"Animals on feed for less than 60 days apparently were not affected.

"Improvement occurred after the onset of cooler weather or with increased levels of supplementary vitamin E in the ration."



Feedlot cattle have a better chance of survival with adequate levels of Vitamin E, according to the results of recent research.

Dr Vanselow said secondary dietary factors could have exacerbated the condition.

"These were high levels of fat (tallow or whole cotton seed), high levels of carbohydrate, and lactic acidosis, rancidity, fungal contamination, nitrites, and ionophores.

"A critical observation was made linking the condition of leg swelling to subsequent deaths from heat stress at three feedlots."

During the two state investigations into leg swelling there were many cattle deaths from heat stress during days of prolonged hot, humid weather in February 1991.

The two feedlots in NSW had both had recent outbreaks of leg swelling that were investigated by the researchers from the Regional Veterinary Laboratory, Armidale.

The feedlot in Queensland had a condition of redness and swelling in the legs in November and December 1990.

Dr Vanselow said a feature of the heat stress deaths was that while hot environmental conditions occurred throughout the region, deaths were confined to feedlot cattle. It apparently did not affect grazing cattle.

"Possibly environmental factors such as lack of shade and crowding did not allow animals to dissipate heat quickly enough. Alternatively the feedlot cattle were different physiologically from grazing cattle."

'A feature of the heat stress deaths was that while hot environmental conditions occurred throughout the region, deaths were confined to feedlot cattle.'

The common factors which link the condition of leg swelling with death from heat stress include:

- Animals less than two months on feed are significantly less at risk.
- The feedlot rations were low in supplementary Vitamin E. Blood vitamin E levels wee low* in these feedlots.
- Damaged blood vessels may affect heat regulation.

Most heat is lost through the skin by either evaporative cooling (sweating) or conduction.

"The body has an ingenious mechanism of bringing hot blood from the body's core to the skin's surface," Dr Vanselow said.

"Small vessels in the skin connect arteries directly to veins. When needed, they open up and blood bypasses the slow capillary network.

"This allows a more rapid flow of blood to the surface and quicker dissipation of heat from the superficial veins.

"This mechanism is most important in the lower limbs, hence the common observation that hot or fevered animals stand in dams.

"In very hot climate conditions it seems likely that the damaged limb vessels will be unable to perform their function in heat regulation with a resultant rise in body temperature which may lead to irretrievable tissue damage throughout the body."

The three feedlots that had heat stress deaths were feeding recommended vitamin E levels based on recommendations from North America.

Dr Vanselow said investigations into leg swelling appeared to indicate these recommendations were unsuitable for Australian summer conditions.

Amery outlines commitment to agriculture

NSW Agriculture Minister, Richard Amery, has outlined the NSW Government's commitment to regulation through the Department of Agriculture.

Mr Amery said key programs would continue and include pesticide monitoring of food, maintaining surveillance for exotic pests and diseases, maintaining rapid identification and response mechanisms for any plant or animal diseases that may get in and efficient and effective quarantine measures in the event of an outbreak of an unwanted disease, weed or other pest.

NSW would quickly move to a more scientifically based "active" animal disease surveillance system, he said.

"This system is more proactive in terms of monitoring and identifying disease problems at the field level in healthy herds, instead of a concentration on passive disease surveillance which relies on information from diagnosis of specimens submitted by producers to laboratories."

Mr Amery also recommitted the Government Veterinary Laboratories at Camden, Orange and Wollongbar to service the State's animal disease and diagnostic needs.

"As I have stated during visits to rural NSW and in consultations with groups like NSW Farmers Association, the veterinary laboratories at Wagga and Armidale will close and the functions they are performing will be carried at Camden, Orange and Wollongbar," Mr Amery said.

"In conjunction with these facilities, commercial laboratories, private veterinary practitioners, Rural Lands Protection Board District Veterinarians and NSW Agriculture veterinary officers will provide a comprehensive and efficient network which will ensure diagnostic services of the highest order while at the same time minimising costs and improving efficiencies."

New guide to he plan winter crop

A publication which is available free of charge to northern farmers provides comprehensive and up-to-date guidelines on how to plan and budget winter cropping enterprises.

Specially written by NSW Agriculture economist at Gunnedah Ian Patrick, "Winter Crop Budgets - Northern Zone 1996" is available free of charge from local NSW Agriculture offices.

The publication contains a feature on developing a grain marketing strategy and explanations of gross margins and enterprise budgets.

Sample budgets are included to guide producers through the easy-to-follow calculations, which will help them determine the profitability of various winter croppi suitable for the property manag

Winter Cro 1996 contains gets on long fallow wheat, for grain, oats canola, chick beans, and cerne for both and western northern crop Additional info crop returns herbices, insec tilizers, and tractor, header costs graingrowers crop managem

Winter Cr 1996 all northern NSW

Moree hereford stud

Moree hereford breeders Guy and Julie Lord have won the northern NSW section of the Seedstock Producer of the Year competition.

The Lords' Gorian hereford stud is based at Wyellan Park, Moree, and its breeding objectives are pitched strongly at the commercial sector.

Its large herd is run under "commercial conditions" and it produces lean, high-growth bulls for commercial and stud breeders Australia-wide.

Judges of the northern competition, NSW Agriculture's Brian Sundstrom and Ebor producer John Cavanagh, found the Lords had worked carefully on their breeding objectives to produce bulls that would breed heavier, leaner steers suitable for feedlots and other markets.

Top northern seedstock producers Guy and Julie Lord of Gorian hereford stud, Wyellan Park, Moree.

Judge John Cavanagh (l- excellence to Lawrenc Toolangatta herefc

The judges were particularly impressed with the way Gorian had pioneered the use and sale of yearling bulls, the high-fertility rates of its large herd, its use of Breedplan, and its extensive progeny testing in commercial herds.

Tambar Springs hereford breeder Lawrence Donoghue of Toolangatta stud was rewarded with a certificate of excellence in the competition.

The judges found the Toolangatta herd was very fertile and its cattle were functional and suited to several markets.

The stud's careful u of performance recor was a feature of operation as was focus on client service.

The Stephens family The Rock hereford stu Coolah won the centr

Country Leader Jan 22, 1996

Lack of vitamin E linked to leg swelling in cattle

A lack of vitamin E has been linked to hind leg swelling and heat stress cattle deaths in feedlots.

In 1990 NSW Agriculture's special veterinary research officer at Armidale Dr Barbara Vanselow began investigating an unexplained condition of leg swelling in feedlot cattle.

Following research in northern NSW and southern Queensland feedlots, Dr Vanselow found that a lack of vitamin E in feedlot cattle was responsible for damage to small blood vessels in the limbs, particularly in the lower hind legs.

It appeared from the results that this damage impairs the body's temperature regulation and results in an inability of the animal to cope with heat stress.

The condition can also occur when fattening cattle for show and when drought feeding.

"The condition is first seen as lameness, fluid swelling of the hock joint, progressing to generalised swelling down to the hoofs and sometimes up the groin," Dr Vanselow said.

"The skin directly above the hoof and around the pastern is reddened, swollen and tight. Affected animals were inclined to have elevated temperatures."

Dr Vanselow's research was carried out at the Armidale Veterinary Laboratory as part of the Cooperative Research Centre for the Cattle and Beef Industry with funding from the Meat Research Corporation.

Comprehensive investigation constantly showed the condition occurred during prolonged hot weather in animals fed low levels of supplementary vitamin E. These same animals had low blood levels of vitamin E.

Blood levels of vitamin E dropped as the number of days on feed increased and animals on feed for less than 60 days were apparently not affected.

"Improvement occurred after the onset of cooler weather or with increased levels of supplementary vitamin E in the ration," Dr Vanselow said.

Petrol prices 'too high'

Tamworth, Narrabri and Coonabarabran are among 18 rural New South Wales areas where motorists were paying too much for petrol, a survey by the NRMA has found.

The survey of 64 NSW country towns found additional transport and storage costs were legitimate reasons for rural petrol prices being above those in the city.

survey showed notional retail margins were almost 12 cents a litre in some areas, and thus the NRMA would step up its campaign to speed industry deregulation.

(The notional retail margin was the actual retail price as recorded by the NRMA less the maximum wholesale price endorsed by the Australian Competition and Consumer Commis-

and unleaded at 81.9cpl), Forbes (LP 83.1cpl and ULP 81.1) and Coonabarabran (LP 82.9 and ULP 80.9).

Dubbo, Parkes, Bega, Wagga Wagga and Griffith were equal fourth, with an average of 81.9clp for LP and 79.9 for ULP.

They were followed by Tamworth and Albury (80.9 LP and 78.9 ULP), Armidale and Narrabri

feeding risks

BEEF producers will be able to safely grain finish their cattle using a new feed additive.

The supplement, virginiamycin, prevents the growth of bacteria responsible for lactic acid accumulation, which can cause illness and death in grazing cattle fed on cereal grain.

One of the developers, Professor Jim Rowe, head of the Department of Animal Science at the University of New England in Armidale, said the registration of virginiamycin would open up a new era of safe grain finishing and drought feeding for cattle producers.

"When the chips are really down, do you use something like this so you can finish cattle grazing in paddocks under the trees with self feeders, or do you lock animals up in confined spaces and full rations in a feedlot?" he said. "Grain feeding under range conditions has tremendous potential in enhancing the clean, green image of Australian beef."

He is supported by prominent Armidale cattleman, Rick Wright, who has called for the recognition of range feeding — grazing supplemented by grain — as a legitimate alternative to full lotfeeding.

Professor Rowe said virginiamycin was completely safe. It remained in the gut and was not absorbed into the animal's fat or muscle. Any waste was excreted with the faeces, where it broke down in a matter of days.

He claimed the registration of virginiamycin for acidosis control in cattle was a world's first.

"You can feed grain you wouldn't otherwise use, such as wheat and triticale, safely under grazing conditions with virginiamycin," he said. "It allows you to feed at the level required to achieve a desired growth rate.

"Say you want to grow some heifers out a half a kilo a day to make a target weight by a certain date, with this feeding system you can put out the desired amount of grain in a self-feeder once a week.

"The animals will eat it all in one or two days and then get the roughage they need from the paddock."

Professor Rowe predicted the additive, to be marketed by Pfizer Animal Health as Eskalin, would allow the majority of beef producers to grain finish.

Finished cattle would be turned off sooner, nutrition and fertility would be improved and graziers would have more control over their production system.

Virginiamycin was developed with funding from the WA Department of Agriculture, the International Wool Secretariat and the Meat Research Corporation.

Robert Taylor, market manager Beef Cattle, Pfiz Health, Dr David Overend and Bill Hiscox, manager c development, Australia, Pfizer and Glenn Davis f Animal Health, at an industry discussion day for Virg

Above: Dr Steve Little, Ridley Agriproducts, Dr James Rowe, UNE, Armidale and Peter Doyle, Pfizer, at an industry discussion day for Virginiamycin.

The Land Feb 8, 1996

Swelling: Vit E deficit blamed

THE lack of vitamin E has been linked by a researcher to hind leg swelling and heat stress cattle deaths in feedlots.

In 1990, NSW Agriculture's special veterinary research officer at Armidale, Dr Barbara Vanselow, began investigating an unexplained condition of leg swelling in feedlot cattle.

Working at the Armidale Regional Veterinary Laboratory, as part of the Cooperative Research Centre for the Cattle and beef industry under MRC funding,Dr Vanselow's research has included feedlots in northern NSW and southern Qld.

The results show a lack of vitamin E in feedlot cattle is responsible for damage to small blood vessels in the limbs, particularly in the lower hind legs. It appears this damage impairs the body's temperature regulation and results in an inability of the animal to cope with heat stress. The condition can also occur when preparing cattle for show and when drought feeding.

"The condition is first seen as lameness, fluid swelling of the hock joint, progressing to generalised swelling down to the hooves and sometimes up to the groin," she said.

The skin directly above the hoof and around the pastern is reddened, swollen and tight. Affected animals were inclined to have high temperatures.

When affected tissue is seen under a microscope there is damage to cells lining small blood vessels which allows leakage of fluid and protein into surrounding tissue. Superficial blood vessels and those around the joints are most severely affected leading to inflammation, haemorrhage and swelling.

The study found in hot climatic conditions it seems likely the damaged limb vessels may be unable to function in heat regulation with a resultant rise in body temperature which could lead to tissue damage throughout the body.

Three feedlots which had heat stress deaths were feeding vitamin E levels based on recommendations from North America. However Dr Vanselow said from her investigations these were unsuitable for Australian summer conditions. She also said naturally stored vitamin E in stored feed in silos was destroyed quickly.

Vitamins E and A are essential supplements in feedlot rations, because no fresh green feed is included.

The vitamins must be balanced in rations as excessive levels of vitamin A can interfere with vitamin E absorption and low levels of vitamin E can affect the stability of vitamin A.