To the Graduate Council:

I am submitting herewith a thesis written by Louie Keith West entitled "The Effect of Vitamins A, D and E Injections on the Blood Plasma of Pregnant Beef Cows and on Calf Viability." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

M. C. Bell

M. C. Bell, Major Professor

We have read this thesis
and recommend its acceptance:

J. W. Holloway

John A. Smalling

Accepted for the Council

L Evans Peck

Vice Chancellor
Graduate Studies and Research

THE EFFECT OF VITAMINS A, D AND E INJECTIONS ON THE BLOOD PLASMA OF PREGNANT BEEF COWS AND ON CALF VIABILITY

A Thesis

Presented for the

Master of Science

Degree

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Louie Keith West

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ABSTRACT

Half of a group of 358 pregnant Angus and Hereford cows at three different experiment stations were injected intramuscularly with 9 ml of a solution containing 4.5 million I.U. of vitamin A, 675,000 I.U. of vitamin D_3 and 450 I.U. of vitamin E. An equal volume of physiological saline was injected into the remainder of the cows. The purpose was to determine the effect of the vitamin injections on plasma beta-carotene, vitamin A, calcium, magnesium, potassium and on calf viability.

Blood samples were taken from the jugular vein immediately prior to the injection, 24 hours later and 28 days later. No noticeable effects were evident due to treatment for any of the plasma values. However, differences in time of sampling were observed for plasma beta-carotene, calcium and potassium.

Differences were detected ($P<.05$) among station source for cow plasma beta-carotene. Values for forage beta-carotene corresponded to plasma beta-carotene at the stations sampled, with those from Alcoa being highest.

Breed differences were not found in plasma beta-carotene and vitamin A levels but the Angus cows were higher ($P<.05$) for plasma calcium and magnesium.

Calves born to the injected cows were scored on viability. More weak calves and fewer healthy calves were born to the vitamin injected cows.

It was concluded that vitamina A, D and E injections given to pregnant beef cows under the conditions of this study do not affect plasma beta-carotene, vitamin A, calcium, magnesium or potassium. Also these vitamin injections when given to pregnant beef cows showed no beneficial effects on calf viability.

CRAVES CREST

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CHAPTER I

INTRODUCTION

Vitamin A is required in the ration of beef cattle since it is not synthesized within the body or digestive tract. Several plant carotenoids (particularly beta-carotene) which are found widely distributed in natural feeds, especially in young, growing plants are converted into vitamin A within the body of the bovine. Weathered or mature roughages as well as most grains and protein supplements are usually low or devoid of vitamin A potency. Vitamin A is also easily oxidized because of its peculiar structure (Pope et al., 1961).

Cattlemen have been concerned with vitamin A deficiencies in areas subject to long periods of drought and poor feed supplies. Fall calving also adds to the problem since a cow must nurse a calf for several months while consuming a ration low in carotene. Vitamin A and carotene are stored in the liver but these stores can be depleted in time. Reports of small calf crops, weak calves, retained placentae, and other non-specific symptons have prompted the use of some form of supplemental vitamin A (Stevens and Williams, 1963). Today, intramuscular injections of vitamin A in combination with vitamins D and E are being given to beef cows in Tennessee and throughout the United States. Vitamin D injections have been shown to increase serum calcium and decrease serum magnesium (Hollis et al., 1977). Vitamin E is usually included in the injection solution as a preservative but it may be beneficial by sparing selenium (Hoekstra et al., 1973).

CHAPTER II

LITERATURE REVIEW

I. VITAMIN A

In 1915, McCollum and Davis first described "fat-soluble A," a growth-promoting factor isolated from fish oils and animal fats. Drummond later suggested the name vitamin A for "fat soluble A" (Goodhart and Shils, 1980). The culmination of years of research came about when vitamin A activity was associated with the yellow carotenes present in plants (Steenbock and Boutwell, 1920).

Vitamin A has many important functions in the body. It maintains the integrity of the epithelium, or covering of the skin and mucous membranes (Wolbach and Howe, 1925; Marsh et al., 1956). It has been demonstrated that vitamin A is essential for the normal functional activity of the reproductive system (Thompson et al., 1964) and for vision (Barnet et al., 1970). Chapman et al. (1964) have recently linked vitamin A with increased gains of cattle during winter trials.

The varying activity of provitamin A and vitamin A substances has made it necessary to establish some guidelines for nomenclature. It has been suggested that the term vitamin A be used as the generic descriptor for all beta-ionone derivatives, other than provitamin A carotenoids, exhibiting qualitatively the biological activity of all-trans retinol. Vitamin A and its provitamins should be expressed in milligram (microgram) all-trans retinol equivalent, while provitamins expressed

separately should be expressed in milligram (microgram) beta-carotene equivalent (Dinning, 1980).

Green plants containing beta-carotene are the primary sources of vitamin A to ruminants. The conversion of beta-carotene to vitamin A and the absorption of preformed vitamin A in the intestinal wall can be improved by the antioxidant effect of vitamin E (Ames, 1962[a]). The beta-carotene content of forages, the conversion of beta-carotene to vitamin A, and the interrelationships of vitamin A with other vitamins and minerals are of importance in beef cattle management.

Effect of Vitamin A Deficiency on Bone

One of the earliest known functions of vitamin A is in the remodeling of the skeleton (Mellanby, 1941). He reported a pattern of bone resorption occurring in deficiency, so that many bones become abnormally thick. In the skull and vertebral column, this may have disastrous secondary effects on the nervous system due to compression of the nervous tissue. Retarded skeletal growth in cattle was noted by Pope et al. (1961) and Moore (1965), as in the closure of the foramen through which the optic nerve passes, thus causing blindness.

Effect of Vitamin A Deficiency on Calving and Incidence of Weak Calves

Since little or no vitamin A is stored in the fetus the gravid cow needs three to four times the minimum requirement of vitamin A (6 to 8 micrograms/kilogram body weight/day of retinol) to produce and maintain normal healthy offspring and a high level of vitamin A and colostrum in milk (Guilbert et al., 1937). The NRC (1976) notes that severe deficiency in the pregnant animal occasionally

results in abortion or birth at term of dead, weak, or blind calves. It has been reported that calves born to dairy cows maintained on green pasture (Fountain et al., 1948) or whose rations were supplemented with high levels of vitamin A (Speilman et al., 1948) during late gestation had increased plasma vitamin A levels at birth. Cattle with plasma carotene levels of 25 micrograms and vitamin A levels of 16 micrograms per 100 ml usually show no signs of vitamin A deficiency (Warkany, 1945). Swanson et al. (1968) recorded a vitamin A level of 5 micrograms per 100 ml of plasma and 1.0 microgram per gram of liver a short time before parturition in vitamin A deficient cows having dead or weak calves. According to Guilbert and Hart (1934), the liver is the major site of carotene and vitamin A storage. Investigations have been made of the effect of level of carotene intake on liver stores of cows at parturition and of their newborn calves. Baker and coworkers (1953) found plasma and liver vitamin A of the calf to be closely associated with the carotene intake of the dam during lactation, but calf levels were also influenced by the liver stores of the cow at parturition.

Dann (1932) suggested that larger amounts of vitamin A are transferred to the calf during lactation rather than in gestation. Colostrum is rich in vitamin A and well absorbed by the offspring so that it helps build a store in the liver (Morton, 1960).

Transport of vitamin A across the placenta has been found by Takahashi et al. (1975) to be well regulated. Transport is maintained with high priority in the presence of maternal deficiency. They reported the primary effect of vitamin A deficiency on fetal growth and

development to reflect on the placenta, with secondary effects on the fetus. The mechanism is not explained but retinal binding protein may be involved since it is known to be bound to the main form (retinal) of vitamin A circulating in the blood (Peterson, 1971).

Recent work by Lotthammer (1979) indicates that beta-carotene has a specific role in cattle fertility, which cannot be replaced by vitamin A. The corpus luteum and ovaries were found to have a high concentration of beta-carotene. They also reported calves from cows fed carotene-deficient diet to have a higher incidence of diarrhea and mortality in the first week of life than calves of the control group (adequate beta-carotene). Cooke (1978) and Friesecke (1978) have also associated beta-carotene deficiencies with lower conception rates, silent heats, delayed ovulation, follicular cysts, delayed and reduced formation of corpora lutea and low blood progesterone levels.

Vitamin A is necessary for the maintenance of the integrity of epithelial tissue. Brocklesby (1960) suggests that when dams have insufficient vitamin A during pregnancy, the endothelium of calves is damaged, leaving the calf open to infection. He reported cases in which the vitamin A status of the dams was directly related to the incidence of bronchial pneumonia in their calves.

II. CAROTENE

Forage Carotene Content and Factors Affecting Its Variation

The wide variation in carotene content of different forages and factors causing variations are important considerations. Cattle

primarily depend on beta-carotene in the roughage for a source of vitamin A except where concentrates supplemented with vitamin A are fed (Hibbs, 1980). Beta-carotene is the most potent and most important provitamin A among the commonly occurring carotenoids such as alpha-carotene, beta-carotene, gamma-carotene and lycopene (Roels, 1970).

Hibbs (1980) reported beta-carotene to be concentrated in the leaves of forages. Therefore, anything causing plant leaf loss will drastically reduce the carotene content of forages. Killing frost and late harvest are associated with low carotene content of corn silage.

Storage of hay results in the loss of carotene so that little remains after a year (Hibbs, 1961). Pfander (1962) related excess heating and mold to carotene loss in hay or silage. Storage losses in the silo are normally slower than in hay because of the exclusion of oxygen.

Drought reduces the content of carotene in pastures (Moon, 1939). Repp and Watkins (1958) determined the regression of precipitation and average mean air temperature upon forage carotene to be highly significant.

O'Dell et al. (1960) have linked the high nitrate and nitrite content of heavily nitrogen fertilized forages with impaired utilization of carotene. It is not known how this interference occurs. Reduced levels of beta-carotene have been found in corn silage heavily fertilized with nitrogen (Klosterman et al., 1963). Smith (1961) suggested the possibility that nitrate exerts a toxic effect through oxidation of carotene, vitamin A, and other such compounds which are susceptible to oxidative destruction.

Carotene Required and the Amount in the Forage

The N.R.C. (1976) has based the international standard for vitamin A on the rate at which the rat converts beta-carotene to vitamin A. Rats convert 1 milligram of beta-carotene to 1,667 I.U. of vitamin A, but it is estimated that 1 milligram of beta-carotene is equal to 400 I.U. of vitamin A with cattle. These conversion factors are based on early studies where mostly fish oils or stabilized natural esters were employed as the source of vitamin A. Ames (1962[b]) stated that the conversion factors should be reduced since such sources of vitamin A are lower in biological potency than the all-trans-isomer. The conversion rate for cattle varies with the breed of animal, individual differences in animals, and level of carotene intake (N.R.C., 1976).

The vitamin A needs of cattle can be met by carotene in feedstuffs. The requirement given by the N.R.C. (1963) for wintering pregnant heifers and cows is 5.5 kilograme of carotene per pound of feed consumed.

III. VITAMIN D

Vitamin D_3 may be produced in the skin by ultraviolet irradiation of 7-dehydrocholesterol. Another source of vitamin D_3 is plants containing vitamin D_2 which is converted to vitamin D_3 in the animal body. The vitamin D_3 is accumulated in the liver where it is hydroxylated to 25-hydrocholecalciferol. The 25-hydroxycholecalciferol proceeds to the kidney where further

hydroxylation to either 1,25-dihydroxycolcalciferol or 24,25-dihydroxy-vitamin D_3 occurs. The 1,25-dihydroxycolcalciferol is the most potent and active form of vitamin D known (DeLuca, 1974).

Relationship of Vitamin D with Calcium and Magnesium

The interrelationships among vitamin D, calcium and magnesium are complex. Most of the attention has been focused on vitamin D and calcium. Magnesium absorption is influenced by the level of calcium in the diet (Behar, 1975). Vitamin D stimulates intestinal calcium absorption according to Coates and Holdsworth (1961), particularly under active intestinal mucosal transport (Kodicek, 1967). Increases in serum calcium and decreases in serum magnesium in steers resulted when cholecalciferol was injected intramuscularly (Hollis et al., 1977) and intravenously (Boling and Evans, 1979). The duration and magnitude of the response was dependent on the method of administration, with the intravenous injection resulting in more pronounced and rapid changes.

IV. VITAMIN E

Vitamin E Effects on Fertility and Calving

Vitamin E has been shown to be concerned with the functions of most of the tissues of the animal body. Evans and Bishop (1922) noted that this fat-soluble factor prevented fetal resorption in rats fed a rancid lard diet. Intrauterine dysfunction has also been noted in the mouse, hamster and guinea pig due to vitamin E deficiency (Goodhart and Shils, 1980). Placental transmission of vitamin E in the rat has been shown to be very small (Mason and Bryan, 1940). Whiting and Loosli

(1948) found that adding tocopherols to the prepartum ration resulted in a highly significant increase in the tocopherol content in the blood plasma of lambs and kids but no increase was observed in pigs. Findings by Gullickson et al. (1949) indicate that placental transmission in the bovine may be greater than in the rat and pig resulting in depletion of the mother instead of the developing fetus. They reported that vitamin E supplementation does not appear to be required for successful reproduction by cattle, since all feeds commonly fed to cattle are relatively rich in vitamin E.

Deficiencies of vitamin E and/or selenium have been shown (Julien et al., 1976; Trinder et al., 1973) to result in increasing the incidence of retained placentas in dairy cows. However, Schingoethe et al. (1978) observed no differences in the number of cases of retained placentas between cows fed low vitamin E stored feed and pastured cows. Another problem associated with this deficiency is the production of calves and lambs with nutritional muscular dystrophy (Young et al., 1961). This degenerative myopathy usually occurs in the first few weeks of life in calves born to cows grazing pastures containing less than 0.1 milligram per kilogram of dry diet (N.R.C., 1976).

Influence of Vitamin E on Vitamin A Stability

The function of alpha tocopherol (vitamin E) as an antioxidant serves to protect vitamin A from oxidation, in feeds, injection solutions and in tissues of the animal body (Smith, 1961). Brocklesby (1960) reported vitamin E increased utilization of vitamin A at high levels of vitamin A intake, while vitamin E had the reverse effect at low intakes of vitamin A. The conversion of beta-carotene to vitamin A

in the intestinal wall and the absorption of preformed vitamin A has been found to be increased by vitamin E (Ames, 1962[a]). Rousseau et al. (1957) observed that tocopherol fed in graded levels over a 12 week period to calves, lambs and pigs appeared to increase plasma vitamin A and decrease liver vitamin A but these differences were not statistically significant.

Rate of growth has been used to measure the effect of vitamin E on the utilization of preformed vitamin A. Rats (Esh, 1949) and calves (Dehority et al., 1961) on diets containing minimal levels of vitamin A showed a suboptimal rate of growth. Supplementation with vitamin E resulted in increased growth rates, as though vitamin E may have a synergistic effect on vitamin A in the diet.

Interaction of Vitamin E and Selenium

The synergistic effect of vitamin E and selenium has been observed in the prevention of liver necrosis of rats (Schwarz and Foltz, 1957), exudative diathesis of chicks (Schwarz et al., 1957) and white muscle disease in lambs (Muth et al., 1959). These diseases were once thought to be due to a dietary deficiency of vitamin E. However, muscular dystrophy in rabbits (Draper, 1957), encephalomacia in chicks (Dam et al., 1957) and resorption of the embryo in pregnant rats (Harris et al., 1958) responded to vitamin E but not to selenium administration even in large doses. These experiments indicated that these two factors have closely allied, yet distinct biochemical roles in metabolism (Hoekstra et al., 1973). They reported selenium to be an integral part of the enzyme glutathione peroxidase which would account for many of the

manifestations of selenium deficiency. No enzyme function of vitamin E has been recorded, yet its vital role as an antioxidant may later prove to be a component of a specific metabolic function.

Geographical variations in selenium have resulted in both toxicity and deficiencies in livestock. Bell and Bacon (1976) reported selenium content of Tennessee forages was variable for hay but uniformly low for corn silage. Deficiencies can be prevented by dietary supplementation or by injectable solutions which normally contain vitamin E along with selenium.

V. MINERALS

Interrelationship of Magnesium and Potassium in Relation to Vitamin D in Ruminants

Potassium in excess has been found to interfere with magnesium utilization in ruminants. High plant potassium content reduces availability of plant magnesium to animals by altering intermediary carbohydrate metabolism (Fontenot et al., 1973). This undoubtedly plays a role in grass tetany and has become a major management problem.

Absorption of magnesium from the gastrointestinal tract is reduced by high potassium intakes (House and Van Campen, 1971), but only when dietary intakes of magnesium are low (Field and Suttle, 1979). The section of the tract where this occurs is not known. Grace et al. (1974) concluded that it would be necessary to evaluate different levels of both magnesium and potassium since the level of magnesium intake affects its site(s) of absorption. Readily available carbohydrate levels should also be considered when evaluating potassium effects on

magnesium in an all forage ration due to increased magnesium absorption directly affected by readily available carbohydrates (Lentz et al., 1976).

CHAPTER III

MATERIALS AND METHODS

I. ANIMALS

A study to determine the effect of an injectable solution of vitamin A in combination with vitamins D and E in pregnant beef cows was performed. The experiment involved 358 Angus and Hereford cows at three experiment stations.

The cows had been randomly divided into breeding groups at the Crossville and Greeneville stations, since this experiment was superimposed on another study, while no breeding groups were considered at the Alcoa station. Breeds included were Angus at the Crossville station, Hereford at the Greeneville station and both Angus and Hereford at the Alcoa station. Alternating cows in the breeding groups and cows at the Alcoa station were injected as they came through the chute in the middle gluteus muscle with 9 ml of a solution containing 4.5 million I.U. of vitamin A, 675,000 I.U. of vitamin D_3 and 450 I.U. of vitamin E.[1] These levels were comparable with those that Beeson et al. (1964) used in 245 kilogram steers and those by Williams and Wheeler (1975) in pregnant beef cows. An equal volume of .85 percent physiological saline was administered in the same manner to the remainder of the cows. The

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[1]Supplied through the courtesy of Hoffman-La Roche, Inc., Nutley, New Jersey.

injections were made between December 11 and December 18, 1979. Blood samples of approximately 25 ml were taken by jugular vein puncture into heparinized syringes immediately prior to the injections, 24 hours later and 28 days later to determine the effects of the treatment on plasma calcium, magnesium, potassium, beta-carotene and vitamin A levels. Calves were born to the injected cows between the middle of January and the first of April and scored on viability: 0 = a healthy calf; 1 = minor calving problem; 2 = weak calf; 3 = weak calf and minor calving problem; 4 = weak calf and mechanical birth assistance; 5 = dead calf.

Analysis

Blood plasma was diluted as needed with double distilled water and analyzed for calcium, magnesium and potassium on an Instrumentations Lab 551 atomic absorption spectrophotometer according to the Atomic Absorption Methods Manual (Emmel et al., 1977).

Vitamin A and beta-carotene determinations and calculations were made on blood plasma as outlined by Sobel and Snow (1947). Colorimetric measurements were made on a Beckman DU II spectrophotometer. The addition of 1 gram antimony trichloride[2] to 100 grams dichloropropanol[3] was made to stabilize the color.

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[2]Fisher Scientific Co., Fair Lawn, New Jersey.

[3]Eastman Kodak Co., Rochester, New York.

II. FORAGES

All cattle had access to forage, dicalcium phosphate, salt and water ad libitum. The amount of each kind of forage varied with pasture availability at each station. When blood samples were obtained, forage samples were taken to determine content of beta-carotene. Forages at the Crossville station sampled included corn silage along with orchardgrass, fescue and bluegrass pastures and mixed clover and grass hay. Alcoa and Greeneville forage samples consisted of corn silage along with orchardgrass and fescue pastures and mixed hay.

Analysis

Forage samples were dried overnight at 60° C and allowed to air equilibrate. The samples were ground in a Wiley mill to pass through a 2 millimeter mesh screen. Analysis of the forage for beta-carotene was carried out according to the procedure outlined in the AOAC (1975) with slight modifications. The actual procedure used for beta-carotene analysis was as follows:

Reagents.

Extractant-hexane-acetone-absolute alcohol-toluene (10+7+6+7) (Fisher Scientific Co.).

Absorbent I--1 + 1 (w/w) silicagel G (J. T. Baker Chemical Co.) and diatomous earth (Fisher Scientific Co.).

Methanolic KOH--40 percent dissolved in MeOH (Fisher Scientific Co.).

Sodium sulfate solution--10 percent (Fisher Scientific Co.).

Elutants--for carotene: hexane-acetone (96 + 4).

Sudan I (Fisher Scientific Co.)--stock solution--dry crystals to constant weight in 70° C vacuum oven. Dissolve .1241 gram in 500 ml acetone-isopropanol (1 + 1).

Working solution--dilute 20 ml stock solution in 500 ml with acetone-isopropanol (1 + 1).

Beta-carotene procedure.

1. Weigh 6 gram of sample into a 100 ml volumetric flask.
2. Add 30 ml of extractant and swirl one minute.
3. Let stand in dark 16 hours.
4. Add 2 ml of 40 percent methanolic KOH and swirl one minute.
5. Let stand in dark one hour.
6. Add 30 ml of hexane, swirl one minute, and dilute to volume with 10 percent Na_2SO_4. Shake vigorously one minute. Let stand in dark one hour.
7. Pipet 20 ml of upper layer onto column.
8. Add carotene elutant as the last of the sample enters absorbent. Continue until carotene band is collected in flask. Keep absorbent covered with solution at all times.
9. Release vacuum and place solution collected in the dark until it reaches room temperature. Dilute to volume with carotene elutant and mix.
10. Set wavelength at 436 (A_{436}), sensitivity at 6, and zero instrument at .03 slit with isopropyl and acetone (1 + 1) blank.
11. Read standard and samples.

Calculations

Calculations were made as follows:

correction factor = $.460/A_{436}$

$$\text{carotene (mg/kg)} = \frac{(\text{reading}(A_{436} \times 454 \times \text{correction factor}))}{196 \times \text{cell length in cm} \times \text{dilution factor}}$$

$$\text{dilution factor} = \frac{\text{g sample} \times \text{ml extracted on column}}{50 \text{ ml upper phase} \times \text{ml final dilution}}$$

III. STATISTICAL ANALYSIS

A split plot design was used in the statistical analysis (Steel and Torrie, 1980) with programs prepared by Blair et al. (1979). The models used were:

$$Y_{ij} = \mu + s_i + t_j + e_{ij}$$

where:

Y_{ij} = dependent variables.

μ = theoretical population mean.

s_i = effect of experiment station, i = 1-3.

t_j = effect of treatment, j = 1-2.

e_{ij} = station x treatment interaction

and

$$Y_{ij} = \mu + s_i + t_j + (st)_{ij} + m_k + (tm)_{jk} + e_{ijk}$$

where:

Y_{ij} = dependent variable.

μ = theoretical population mean.

s_i = effect of experiment station, i = 1-3.

t_j = effect of treatment, j = 1-2.

$(st)_{ij}$ = experiment station x treatment interaction.

m_k = effect of sampling time.

$(tm)_{jk}$ = treatment x sampling time interaction.

e_{ijk} = experiment station x sampling time interaction + experiment station x treatment x sampling time interaction.

CHAPTER IV

RESULTS AND DISCUSSION

I. PREGNANT BEEF COWS

Time of Sampling

Plasma vitamin A in both the saline and vitamin injected groups decreased ($P<.05$) following injection (Table 1). A decrease in plasma vitamin A prior to calving has been reported in the literature (Sutton et al., 1945). It was observed by Rowlands et al. (1975) that a drop in the total protein concentration in the blood (including albumin and globulin) occurs weeks prior to calving. This decline could lead to a drop in plasma vitamin A before parturition, since vitamin A is transported in the blood by certain proteins in the albumins and globulins. The injection of 4.5 million I.U. of vitamin A did not prevent the drop in plasma vitamin A.

There was a significant ($P<.05$) increase in plasma beta-carotene 24 hours after initial blood samples were taken and then a significant ($P<.05$) decrease 28 days later (Table 1). Stahr (1977) reported a standard range of serum carotene in cows to be from 25 to 950 micrograms per 100 ml. The variations in plasma beta-carotene and vitamin A would suggest the examination of liver vitamin A to more accurately determine the status of the animal. Pope et al. (1961) determined that plasma carotene and liver vitamin A are

TABLE 1

EFFECT OF COW VITAMIN INJECTIONS ON PLASMA BETA-CAROTENE, VITAMIN A, CALCIUM, MAGNESIUM AND POTASSIUM USING LEAST SQUARE MEANS

Treatment[+]	Days After Injections		
	0	1	28
	plasma beta-carotene in μg/100 ml		
saline	155.4[a]	190.8[b]	141.6[a]
vitamins	153.5[a]	203.4[b]	137.0[a]
	plasma vitamin A in μg/100 ml		
saline	42.9[a]	37.0[a]	37.1[a]
vitamins	47.7[a]	37.6[a]	39.0[a]
	plasma calcium in mg/100 ml		
saline	10.9[a]	11.6[b]	11.1[ab]
vitamins	10.8[a]	11.7[b]	11.2[ab]
	plasma magnesium in mg/100 ml		
saline	2.1[a]	2.2[a]	2.2[a]
vitamins	2.1[a]	2.2[a]	2.2[a]
	plasma potassium in mg/100 ml		
saline	19.9[a]	22.0[b]	21.6[b]
vitamins	19.9[a]	22.1[b]	21.8[b]

[+]Differences among treatments were not statistically significant ($P > .05$).

[ab]Values in the same row having different superscripts are significantly different (plasma beta-carotene $P < .05$; plasma calcium $P < .05$; plasma potassium $P < .01$).

significantly associated only after a long period of high carotene intake. Conversely, plasma and liver vitamin A appear to be significantly correlated only after a long depletion period.

It is possible that the filling of the mammary with colostrum, which is rich in globulin and albumin, removes carotene and vitamin A from circulating blood. Sutton and Soldner (1943) observed normal levels of approximately 16 micrograms of plasma vitamin A and 400 micrograms of plasma carotene per 100 ml of plasma in 18 dairy cows during a winter feeding period, until about one week prior to parturition. The average levels of carotene and vitamin A had dropped within three days of parturition to 235 micrograms and 8.2 micrograms per 100 ml, respectively.

Significant changes in plasma calcium ($P<.05$) and plasma potassium ($P<.01$) at different times of sampling were found (Table 1). This could be explained by different dietary intakes of these elements.

The effect of the vitamin injections on blood plasma values measured at the three experiment stations tested is shown in appendix, Tables 5, 6, 7. Although differences among them could not be tested statistically, mean plasma levels of beta-carotene, vitamin A, calcium, magnesium and potassium station-treatment-time subclasses are given. These tables tend to indicate that a more accurate method for determination of the beta-carotene and vitamin A status of pregnant beef cows is needed.

Treatment Effect

Differences between saline and vitamins A, D and E injected cows were not significant ($P>.05$) for any of the plasma values tested (Table 1). This is in accordance with work by Bell et al. (1979) who reported no effect ($P>.05$) of vitamins A, D and E injections on plasma calcium, magnesium and vitamin A in 50 pregnant beef cows compared with 48 controls. This would question the indiscriminate use of vitamins A, D and E injections for beef cows in Tennessee under these wintering conditions.

Differences Among Stations

A statistical analysis of plasma beta-carotene (Table 2) indicated the samples from the Alcoa station were higher ($P<.01$) than those from the cows at the other two stations tested. The beta-carotene in the plasma corresponded to the amount of beta-carotene in the forages at all three stations. These forage levels of 2.0 to 2.8 milligrams per kilogram were lower than the N.R.C. (1963) recommended level of 5.5 milligram per kilogram for a wintering mature pregnant beef cow. However, Beeson et al. (1964) indicates that it takes 210 days to deplete a single intramuscular injection of 6 million I.U.

Plasma calcium was higher ($P<.05$) in cows at the Alcoa and Crossville stations when compared to cows at the Greeneville station. The cows at the Alcoa station approached significance ($P>.05$) in lower plasma magnesium when compared to the cows from other stations tested (Table 2). Bell et al. (1979) also reported lower plasma magnesium and higher plasma calcium from cows at the Alcoa station compared to other

TABLE 2

STATION VARIATIONS IN ANIMAL PLASMA AND FORAGE ANALYSIS FOR ALL SAMPLING TIMES

Item	Stations		
	Alcoa	Crossville	Greeneville
No. of animals	89	145	124
Plasma beta-carotene μg/100 ml	194.7[a]	151.3[b]	152.0[b]
Plasma vitamin A μg/100 ml	38.1	38.4	44.6
Plasma calcium mg/100 ml	11.5[a]	11.6[a]	10.6[b]
Plasma magnesium mg/100 ml	2.1	2.2	2.2
Plasma potassium mg/100 ml	21.8	21.2	20.8
Forage beta-carotene mg/kg	2.8	2.0	2.5

[ab]Values in the same row having different superscripts are significantly different (plasma beta-carotene $P<.01$; plasma calcium Alcoa vs. Greeneville $P<.05$, Crossville vs. Greeneville $P<.01$).

stations tested. The high plasma calcium to magnesium ratio has been associated with hypomagnesemic tetany syndrome (Boling and Evans, 1979). This could explain the higher incidence of grass tetany at the Alcoa station.

Breeds

The only significant ($P<.05$) breed differences observed were in plasma calcium and magnesium (Table 3). It has long been recognized that variations in the amount of circulating carotene occur in different breeds of dairy cattle. Pope et al. (1961) concluded that little difference in plasma carotene and vitamin A occurs between Angus and Hereford breeds. This was found to be true in this study.

Calf Viability

Saline injected cows gave birth to more healthy calves and fewer weak calves than the vitamin injected cows (Table 4). Each station tested recorded more healthy calves born to saline injected cows than vitamin injected cows (Appendix, Table 8). This tends to disagree with Williams and Wheeler (1975) who reported that cows injected with vitamin A had more live calves at weaning (87.5 percent) than cows not injected (81.2 percent). These differences were not significant ($P>.05$) but were consistent throughout three trials.

Not all of the calves born to the vitamin and saline injected cows were scored which could have contributed to the differences in the two groups. However, the larger number of viable calves born to saline injected cows would question the need for vitamins A, D and E injections in pregnant beef cows in Tennessee.

TABLE 3

LEAST SQUARE MEANS AMONG BREEDS AT THE ALCOA EXPERIMENT STATION

Item	Breeds	
	Angus	Hereford
No. of animals	54	35
Plasma beta-carotene μg/100 ml	191.9	199.1
Plasma vitamin A μg/100 ml	38.4	37.4
Plasma calcium mg/100 ml	11.6[a]	11.2[b]
Plasma magnesium mg/ 100 ml	2.2[a]	2.0[b]
Plasma potassium mg/100 ml	21.9	21.8

[ab]Values significantly different from other values in the same line not bearing the same superscript ($P<.05$).

TABLE 4

VIABILITY OF CALVES AT BIRTH AS AFFECTED BY INJECTIONS INTO PREGNANT BEEF COWS

Calf Score	Injections	
	Saline	Vitamins
0 = healthy calf	138	128
1 = minor calving problem	4	6
2 = weak calf	12	21
3 = weak calf and minor calving problem	1	1
4 = weak calf and mechanical birth assistance	4	4
5 = dead calf	7	7
Total	165	167

CHAPTER V

SUMMARY

No significant effect on plasma beta-carotene, vitamin A, calcium, magnesium or potassium was found following the intramuscular injection of vitamina A, D and E. High levels of vitamin A did not prevent a decrease in plasma vitamin A prior to calving. Variations in plasma beta-carotene and vitamin A indicates that liver stores of vitamin A might be a more reliable indicator of the vitamin A status of the animal (Pope et al., 1961).

Station differences in forage beta-carotene were reflected in the plasma beta-carotene of the cows consuming the forages. Also the cows at one station which have a higher incidence of grass tetany were found to have a higher plasma calcium to magnesium ratio when compared to the other two stations tested.

Breed differences for plasma beta-carotene and vitamin A were not statistically significant. However, the Angus breed was significantly higher in plasma calcium and magnesium than the Hereford breed.

More weak calves and fewer healthy calves were born to the vitamin injected cows than to the controls injected with physiological saline. This indicates that vitamin A, D and E injections in pregnant beef cows may not be needed under Tennessee conditions employed in this study.

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APPENDIX

APPENDIX

TABLE 5

EFFECT OF COW VITAMIN INJECTIONS AT THE ALCOA STATION ON PLASMA BETA-CAROTENE, VITAMIN A, CALCIUM, MAGNESIUM AND POTASSIUM USING LEAST SQUARE MEANS

Plasma	Days After Injection		
	0	1	28
Saline			
Beta-carotene μg/100 ml	158.8	207.4	210.9
Vitamin A μg/100 ml	44.3	41.7	28.2
Calcium mg/100 ml	11.2	11.9	11.4
Magnesium mg/100 ml	2.0	1.9	2.6
Potassium mg/100 ml	19.9	23.1	22.7
Vitamin			
Beta-carotene μg/100 ml	180.1	203.0	208.3
Vitamin A μg/100 ml	43.0	43.2	27.8
Calcium mg/100 ml	11.1	11.8	11.2
Magnesium mg/100 ml	1.9	1.8	2.5
Potassium mg/100 ml	19.7	22.6	23.1

TABLE 6

EFFECT OF COW VITAMIN INJECTIONS AT THE CROSSVILLE STATION ON PLASMA BETA-CAROTENE, VITAMIN A, CALCIUM, MAGNESIUM AND POTASSIUM USING LEAST SQUARE MEANS

Plasma	Days After Injection		
	0	1	28
Saline			
Beta-carotene μg/100 ml	141.5	175.4	134.0
Vitamin A μg/100 ml	36.4	29.2	49.5
Calcium mg/100 ml	11.0	12.0	11.6
Magnesium mg/100 ml	2.3	2.3	2.2
Potassium mg/100 ml	20.0	21.6	22.3
Vitamin			
Beta-carotene μg/100 ml	138.2	199.2	120.3
Vitamin A μg/100 ml	36.5	28.1	50.0
Calcium mg/100 ml	10.9	12.2	11.9
Magnesium mg/100 ml	2.3	2.3	2.1
Potassium mg/100 ml	19.5	22.0	21.9

TABLE 7

EFFECT OF COW VITAMIN INJECTIONS AT THE GREENEVILLE STATION ON PLASMA BETA-CAROTENE, VITAMIN A, CALCIUM, MAGNESIUM AND POTASSIUM USING LEAST SQUARE MEANS

Plasma	Days After Injection		
	0	1	28
Saline			
Beta-carotene μg/100 ml	175.4	197.9	93.9
Vitamin A μg/100 ml	52.5	44.2	28.2
Calcium mg/100 ml	10.6	10.9	10.3
Magnesium mg/100 ml	2.1	2.4	2.0
Potassium mg/100 ml	19.9	21.6	19.9
Vitamin			
Beta-carotene μg/100 ml	151.2	210.3	102.9
Vitamin A μg/100 ml	66.4	46.6	32.8
Calcium mg/100 ml	10.6	10.9	10.2
Magnesium mg/100 ml	2.1	2.4	2.0
Potassium mg/100 ml	20.7	21.9	20.9

GRAMES CREST

TABLE 8

VIABILITY OF CALVES AT BIRTH AS AFFECTED BY INJECTIONS INTO BEEF COWS AT ALCOA, CROSSVILLE AND GREENEVILLE EXPERIMENT STATIONS

Calf Score	Station		
	Alcoa	Crossville	Greeneville
Saline			
0 = healthy	40	59	39
1 = minor problem	2	0	2
2 = weak	0	5	7
3 = weak and minor problem	0	0	0
4 = weak and mechanical assistance	0	2	2
5 = dead	3	0	4
Total	45	66	54
Vitamin			
0 = healthy	36	57	35
1 = minor problem	5	0	1
2 = weak	1	1	19
3 = weak and minor problem	0	0	1
4 = weak and mechanical assistance	0	4	0
5 = dead	2	3	2
Total	44	65	58

CRANESE CREST

VITA

Louie Keith West was born June 24, 1956 in Carthage, Tennessee. He grew up on a 212 acre farm owned by his parents, near Pleasant Shade, in Smith County, Tennessee. He attended elementary and high school there and later enrolled at The University of Tennessee at Knoxville. He received a Bachelor of Science degree in agriculture on June 9, 1978. In September of 1979 he returned to pursue a Master of Science degree in agriculture.