VITAMIN A STATUS OF EWES AND LAMBS GRAZING NITROGEN FERTILIZED ORCHARDGRASS PASTURES

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SUMMARY

Experiments described were conducted to define the vitamin A status of ewes and lambs grazing orchardgrass fertilized at various levels of N or N plus micro-elements. Four fertilizer treatments (56 kg N/ha; 168 kg N/ha; 504 kg N/ha; 504 kg N/ha + Co, Cu, Mo, Zn and S) were applied to replicated hay and pasture plots of Potomac orchardgrass on a Gilpin silt loam soil. Ewe lambs were allocated randomly to fertilizer treatment groups and maintained on pasture and hay from 1967 to 1971.

Forage nitrate and carotene levels increased with increasing levels of N fertilizer, but fertilizer level did not affect ewe blood carotene, blood vitamin A or liver vitamin A levels. Overall average values were 0.06% NO$_3$-N and 127 mg carotene per kilogram of forage DM, 12.3 mcg carotene and 26.7 mcg vitamin A per 100 ml of blood, and 92.5 mcg vitamin A per gram of fresh liver. Correlation analysis of animal data (blood carotene, blood vitamin A, and liver vitamin A) with forage components (nitrate and carotene) indicated that while some co-efficients were significant (P<.05), there was little association of animal and plant factors. Blood and liver vitamin A tended to be higher in animals grazing pastures treated with high levels of N.

No measurable vitamin A was present in livers of newborn lambs, but large liver concentrations of carotene were found in newborn lambs in all fertilizer treatment groups. There was no difference in these levels among fertilizer treatments.

(Key Words: Vitamin A, Sheep, Orchardgrass Pastures, Nitrogen Fertilization.)

INTRODUCTION

Recently vitamin A deficiency signs have been described in animals receiving diets previously thought to supply adequate levels of the vitamin (Mitchell, 1960; Jordan et al., 1961; Neumann, 1961; Newland and Deans, 1964; Smith et al., 1964; Hinds, 1965). A number of naturally occurring compounds are suspected of destroying vitamin A precursors in the gut (Klatte et al., 1964), decreasing the efficiency of carotene conversion in the wall of the small intestine (Reddy and Thomas, 1962), impairing vitamin A transport and storage mechanisms, or increasing the rate of vitamin A mobilization from the liver. Intensive management of livestock and pasture has led to decreased intake of tocopherols and some trace elements by grazing animals (Blaxter, 1952; McGillivray, 1952; O'Donovan et al., 1966). On the other hand, with increasing use of nitrogen fertilizer, levels of nitrate in the grazing animals' diet have generally been raised.

This study was conducted to determine the vitamin A status of ewes grazing Potomac orchardgrass (Dactylis glomerata) or fed orchardgrass hay receiving levels of N fertilizer, or N + micro-elements.

EXPERIMENTAL PROCEDURE

Materials and management procedures have been described previously (Reid et al., 1974; Horn et al., 1974). Fertilizer treatments of pastures and hay crops were as follows: (1) low nitrogen (LN)-56 kg N/ha; (2) medium nitrogen (MN)-168 kg N/ha; (3) high nitrogen (HN)-504 kg N/ha; and (4) high N plus micro-elements (HT)-504 kg N/ha and Co, Cu, Mo, Zn and S at...
the rate of .67, 3.36, 1.34, 6.87 and 13.49 kg/ha, respectively.

Forage for carotene analysis was sampled at approximately monthly intervals at a number of sites within each pasture to ensure a representative sample. Grass was “quick frozen” on dry ice, then stored at −5°C. At the time of analysis, each sample was mixed, subsampled, weighed and finely chopped. Carotene was isolated by column chromatography according to A.O.A.C. (1960) methods. Crystalline beta-carotene was used as a reference substance.

Forage samples for nitrate analysis were collected at approximately monthly intervals during the 4 years of the study except when available forage levels were so reduced as to prevent sample collection. Nitrate determinations were conducted according to the methods of Bremner and Keeney (1966) as modified for plant tissue analysis.

In May, 1967, 72 Western ewe lambs were randomly allocated to eight groups. Two ewes within each group were selected at random for liver biopsy and blood sampling. The groups were released on the eight grazing plots and the following measurements were made throughout the first year at approximately monthly intervals: (1) blood and liver carotene and vitamin A concentrations; (2) forage carotene, nitrate and total nitrogen levels. Liver biopsy samples were taken each summer in subsequent years and again at the conclusion of the trials in September, 1971, when six ewes per treatment group were slaughtered for tissue analysis.

Ewe liver samples were taken by the aspiration biopsy technique of Bone (1954). Samples were immediately weighed and placed on dry ice, then transferred to a freezer and stored at −5°C. Analysis of liver samples for vitamin A was conducted by the method of Ames (1954) as modified by F. C. Hinds (personal communication). Frozen liver (.3 to .5 g) was ground in a mortar with three to four times the sample weight of anhydrous sodium sulfate. The sample was then transferred quantitatively to a glass-stoppered 50 ml centrifuge tube, and 30 ml of anhydrous ethyl ether were added. The tube was shaken for 2 min and stored at −5°C under nitrogen gas for 24 hours. The ether extract was transferred to a stoppered tube after filtering through anhydrous sodium sulfate, and the ground tissue was rinsed with an additional 20 ml of ethyl ether. The ether extract was immediately redissolved in chloroform, and vitamin A was estimated by reaction with Carr-Price reagent (Carr and Price, 1926). There was no carotene apparent in ether extracts from ewe liver samples. All-trans vitamin A acetate dissolved in chloroform was used as a reference substance.

Blood serum was obtained from ewes by centrifugation of jugular blood. Carotene-vitamin A determinations in serum were conducted by the method of Kimble (1939) as described by Allport and Keyser (1957).

Ewes were bred to Dorset or Dorset-cross rams in late October of 1967. Liver samples were not collected during late pregnancy, but a biopsy was performed after lambing, before the ewes were returned to the pasture. One animal from each set of newborn twin lambs (at least six lambs per fertilizer treatment) was removed from the dam immediately after birth, before suckling. These lambs were slaughtered and samples of liver and blood were taken for vitamin A and carotene analysis.

RESULTS AND DISCUSSION

Forage Samples. Mean forage nitrate values are shown in table 1. Levels attained on the high-nitrogen fertilized plots were never above the .21% NO₃-N level suggested as potentially toxic (Wright and Davison, 1964; Gordon et al., 1962) although this concentration was occa-

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ₐ,b,c,d Values in the same column with different subscript are significantly different (P<.05).
sionally exceeded at early growth stages of the herbage (Reid et al., 1974). Subclinical effects on growth and vitamin A metabolism have been reported in animals consuming as much as .21% NO₃-N (Zimmerman et al., 1962; Cline et al., 1963; Jordan et al., 1963; Smith et al., 1964; Hinds et al., 1965, 1967). No harmful effects of nitrate have been reported in animals consuming nitrate in amounts which compare with those found in the present work.

Seasonal changes were similar for all fertilizer treatments, with highest concentrations of nitrate in early August and late November. Notably higher concentrations of nitrate occurred in high-nitrogen fertilized herbage throughout the grazing season. Analysis of variance of data from plots receiving increments of nitrogen (LN, MN and HN) showed a highly significant (P<.01) increase in herbage nitrate with increasing levels of nitrogen fertilizer application. Heterogeneity of variance made the comparison of HN and HT treatments impracticable.

Mean grass carotene values for the four fertilizer treatments during the 1967 grazing season are shown in figure 1. A marked increase in carotene content of forages receiving high levels of nitrogen (HN and HT) occurred in the fall, while no corresponding increase in LN and MN herbages was noted. The fall increase in carotene content followed the second application of nitrogen on HN and HT plots. Similar carotene responses to nitrogen fertilization have been reported by Moon (1939), Holmes (1949), Smith et al. (1964), but not by Hinds et al. (1967) who noted that little or no increase in carotene accompanied a split application of 111 kg N/ha to orchardgrass plots at the Illinois station during the 1964 grazing season. Analysis of variance revealed a highly significant (P<.01) increase in grass carotene content associated with increasing levels of nitrogen fertilizer application.

Blood Samples. Seasonal changes in serum vitamin A and carotene levels in the ewes were not different (P>.05) among nitrogen fertilizer treatment groups during the grazing season. Serum vitamin A values averaged across treatments, for all ewes, are shown in figure 2. A comparison of values in figures 1 and 2 indicated that blood vitamin A values tended to reflect carotene concentrations in pasture herbage during the entire grazing season on the high-nitrogen fertilized forage only. Simple correlations were not significant (P<.05), however, probably because of high variability associated with all blood values. All ewe blood values were within the normal range as reported by Pope et al. (1949).

Liver Samples. Trends in ewe liver vitamin A levels during the 1967 grazing season are shown in figure 3. While analysis of variance indicated no significant differences (P>.05) among nitrogen fertilizer treatments, increased levels of nitrogen fertilization tended to be associated with higher liver stores of the vitamin. Vitamin

![Figure 1](image1.png)  
Figure 1. Mean forage carotene content for fertilized orchardgrass pastures during the 1967 grazing season.

![Figure 2](image2.png)  
Figure 2. Overall mean serum vitamin A values for ewes grazing orchardgrass pastures over the 1967 grazing season.
A levels in the liver were shown previously to be a reliable indicator of vitamin A status (Dicks et al., 1957; Jones et al., 1962), but it should be noted that all liver values presented were within the "normal" range. Liver vitamin A levels (figure 3) would suggest that ewes grazing HN and HT pastures went into the winter with high liver stores of vitamin A. Among the reasons for undertaking the present investigation were conflicting reports suggesting that nitrate in the diet of animals decreased their ability to maintain adequate liver stores of vitamin A (Mitchell et al., 1955; O'Dell et al., 1960; Sokolowski et al., 1960; Hale et al., 1961; Hatfield et al., 1961; Jordan et al., 1961; Zimmerman et al., 1962; Olson et al., 1963; Weichenthal et al., 1963). While such reports are numerous, most have been inconclusive and indeed, other workers have been unable to find effects of dietary nitrate on liver vitamin A storage by ruminants (Pope et al., 1961). In addition, while most reports of nitrate interference came from the Illinois station in studies with corn silage and high concentrate diets as well as pasture herbage, Hinds (1965) has reported that, at least in grazing cattle, there appeared to be no predictable effect of dietary nitrate in different years. To determine whether there were significant relationships between variables examined in this study, simple correlation coefficients between forage nitrate, forage carotene, blood carotene, blood vitamin A, and liver vitamin A were calculated. Although individual coefficients were statistically significant, the magnitude of the correlations, and their consistency among treatments, did not indicate the existence of any strong relationship between forage and animal components. Since all analyses of variance revealed significant (P<.01) differences for all parameters among sampling dates, grass nitrate was adjusted in covariance analysis and the differences due to date of sampling were re-examined. Presumably, if measurements of sheep vitamin A status were dependent upon grass nitrate level, data differences would no longer be obvious in the adjusted values. The occurrence of significant date differences among treatments with grass nitrate adjusted leads one to conclude that carotene utilization is, in fact, independent of the concentration of nitrate in the herbage, and that rather it is controlled by some other factor, or factors, which itself varies during the grazing season.

In 1968 and 1969, liver samples were collected by biopsy in May, July and October and samples from whole liver were also taken from ewes slaughtered at the conclusion of the trials in September, 1971. Average values (figure 4)
indicated that while fertilizer treatment was not related to liver vitamin A concentration, vitamin A did increase with advancing age of the ewe.

No vitamin A could be found in liver samples collected from newborn lambs. Similar results have been reported not only for lambs, but for several newborn mammals (Wise et al., 1948). While levels of the vitamin were low in newborn lamb liver, carotene levels in the liver tissue were variably high. Carotene values ranged from 3 to 31 mg/g of fresh liver in the lambs slaughtered immediately after birth but before suckling. Fertilizer treatment had no apparent effect on the liver carotene levels.

Further investigation of the lamb carcasses revealed enlarged thyroid glands. Thyroid studies have been described in detail elsewhere (Horn et al., 1974), but it is important to note here that one sign of hypothyroidism is hypercarotenemia (Pitt-Rivers and Trotter, 1964).

**LITERATURE CITED**


