RELATIONSHIP BETWEEN NYCTALOPIA AND PLASMA VITAMIN A LEVELS AND THE EFFECT OF VITAMIN A SUPPLEMENTATION IN BEEF CALVES

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SUMMARY

Two groups of Hereford calves with a prior history of low plasma vitamin A levels and nyctalopia were fed sorghum grain *ad lib.* from 16 to 20 weeks of age. One group was supplemented with vitamin A by drenching.

Plasma vitamin A levels of 8 μg/100 ml or less were associated with nyctalopia. Calves recovered from nyctalopia within a week of vitamin A administration.

A difference in light intensity sufficient to influence the onset of nyctalopia was evident in a time interval of 10 min between successive runs through a maze.

There were no significant differences in blood haemoglobin, packed cell volume and white cell count between vitamin A deficient and supplemented calves.

Grain consumption was higher in the supplemented group, being 5·49 lb per head per day compared with 4·20 lb for the control group. The mean body-weight gain was 5·5 ± 0·8 and 3·1 ± 0·8 lb per week for the supplemented and control group respectively.

I. INTRODUCTION

Data on the depletion of liver vitamin A reserves have been obtained as part of drought feeding studies with beef cattle fed for survival on crushed sorghum grain containing virtually no carotene (Ryley, Gartner, and Morris 1960; Ryley and Gartner 1962*a*). On the basis of these experiments both with maiden yearling heifers and with pregnant and lactating cattle, Gartner and Ryley (1962) concluded that the greater the initial vitamin A reserve in the liver the less the chance that this reserve may be depleted completely in periods of up to six months on rations containing negligible carotene. Initial vitamin A levels of the order of 300 μg/g enabled Hereford heifers to carry their calves for the last three months of gestation and nurse them for the first three months of lactation and still maintain adequate reserves on all-sorghum grain rations. These workers also showed a positive relationship between liver vitamin A reserves and vitamin A concentration in butterfat, indicating that in this method of drought feeding either adequate initial reserves of vitamin A of the dams or vitamin A supplementation to dams or calves is necessary to ensure the immediate vitamin A requirement of the suckling calf.

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In the experiment concerning the feeding of restricted amounts of crushed sorghum grain to cattle for 24 weeks during late pregnancy and early lactation, calves had access to the grain *ad lib.* for a period of six weeks prior to weaning (Ryley and Gartner 1962*a*). They were then weaned and fed a sole ration of crushed sorghum grain *ad lib.* for six weeks as part of an experiment on the early weaning of beef calves (Ryley and Gartner 1962*b*). During this period calves had received some vitamin A from milk, but negligible amounts of carotene from the grain. Two calves died after weaning and low levels (<1 μg/g) of vitamin A were found in their livers. At the conclusion of early weaning some calves had low concentrations of plasma vitamin A (<4 μg/100 ml) and nyctalopia. The opportunity was taken therefore to examine in greater detail the relationship between nyctalopia and plasma vitamin A levels and the effect of vitamin A supplementation. The results of this study are reported in this paper.

II. MATERIALS AND METHODS

(i) *Experimental Yards and Facilities.*—The experiment was conducted in yards described by Ryley, Gartner, and Morris (1960). The perimeter outside the yards, approximately 1·5 ft wide, was kept bare of vegetation.

(ii) *Experimental Animals.*—These were the calves born during an experiment in which pregnant and lactating cattle were fed for survival on restricted amounts of crushed sorghum grain under drought feeding conditions (Ryley and Gartner 1962*a*). Calves were supplemented at an average age of six weeks (28–57 days) with crushed sorghum grain fed *ad lib.* in a creep. They were weaned four weeks later, retained in yards and fed crushed sorghum grain *ad lib.* for a further six weeks (Ryley and Gartner 1962*b*). At the end of this period there was evidence of nyctalopia and low plasma vitamin A levels (<4 μg/100 ml.) in some calves. The experiment reported in this paper commenced when the average age of the calves was 16 weeks.

(iii) *Sorghum Grain.*—A single consignment of crushed *Sorghum vulgare* L. grain (Alpha variety) supplemented with 1 per cent. added limestone was used as the sole source of feed to the calves.

(iv) *Test for Nyctalopia.*—The calves were individually driven through a maze at dusk to test for the incidence of night blindness. At each test it was ensured that some of the last-driven animals avoided the obstacles. This established that sufficient light was present for animals with normal vision to negotiate the maze successfully.

(v) *Body-weight.*—The calves were individually weighed on a cattle-weighing scale calibrated in 2-lb divisions.

(vi) *Method of Chemical Analyses.*—Vitamin A in plasma was determined by the method of Peirce (1945). Haemoglobin was measured in heparinized blood by the method of Donaldson *et al.* (1951) and packed cell volume (P.C.V.)

by essentially the method of Wintrobe (1947), using a relative centrifugal force of approximately 2067*g*. White cell count was determined as described by Dacie (1956).

III. EXPERIMENTAL

(i) *Design*.—The calves were divided into two comparable groups of eight on the basis of sex, body-weight, plasma vitamin A and presence or absence of nyctalopia. One group was supplemented with vitamin A by drenching with 15 g high-potency fish oil (25,000 I.U./g), the other group being retained as a non-supplemented control. The calves were retained in these groups and fed crushed sorghum grain *ad lib.* for a further four weeks (from 16 to 20 weeks of age). The control calves were then drenched with vitamin A at the same rate and all animals were removed from the yards and turned out to graze *Paspalum dilatatum* pastures.

(ii) *Observations and Recordings*.—Calves were weighed at weekly intervals between 8 a.m. and 8.30 a.m. Water and feed were available *ad lib.* at all times. Weekly group feed intakes were calculated from the amount of feed fed and residues remaining on the weekly weighing days. Animals were observed daily for evidence of scouring. All animals were bled for the determination of haemoglobin, P.C.V. and plasma vitamin A and tested for the incidence of nyctalopia prior to allocation into experimental groups (pretreatment) and after four weeks of all-grain feeding (final). In addition, white cell counts were made at the second bleeding. The calves were driven through a maze again one week after the control group had been supplemented with vitamin A.

IV. RESULTS

The pretreatment and after-four-weeks (final) body-weights, plasma vitamin A levels and incidence of nyctalopia of the calves are shown in Table 1.

(i) *Plasma Vitamin A*.—In the initial plasma vitamin A analyses, carotene could not be detected in the blood of these calves. However, the final samples from the control group did show the presence of carotene in some samples. An inescapable conclusion is that some animals from the control group must have had access to limited green feed. This is reflected also in the increased plasma vitamin A levels in four of the control calves at the final sampling.

(ii) *Nyctalopia*.—Although there appeared to be some difference between animals in the degree of night blindness, this could not be measured quantitatively and nyctalopia was recorded as either present or absent. In this environment in midsummer, night blindness could only be detected approximately one hour after sunset. In fact, the difference in light intensity sufficient to influence the onset of nyctalopia was evident in a time interval of only 10 min between successive runs through the maze. All animals in the control group successfully negotiated the maze when driven through again one week after the control group was supplemented with vitamin A at the conclusion of the experiment. No ophthalmoscopic examinations were made for papillary oedema, but visual eye examinations initially and four weeks later showed no external lesions.

TABLE 1

INITIAL AND FINAL BODY-WEIGHTS, PLASMA VITAMIN A LEVELS AND INCIDENCE OF NYCTALOPIA OF HEREFORD CALVES FED SORGHUM GRAIN *ad lib.* WITH AND WITHOUT VITAMIN A SUPPLEMENTATION

Supplemented						Control					
Body-weight (lb)		Plasma Vitamin A (μg/100 ml)		Nyctalopia		Body-weight (lb)		Plasma Vitamin A (μg/100 ml)		Nyctalopia	
Pretreatment	Final	Pre-treatment	Final	Pre-treatment	Final	Pretreatment	Final	Pre-treatment	Final	Pre-treatment	Final
162	196	<4	23	+	−	155	144	<4	<4	+	+
159	188	13	29	−	−	159	172	13	10	−	−
94	106	17	27·5	−	−	98	115	17	21*	−	−
148	171	8·5	22	−	−	162	187	7	17·5*	−	−
123	140	8	21	+	−	92	102	5·5	7·5†	+	+
122	141	7	33	+	−	139	160	9·5	8†	−	+
149	175	10	23	−	−	140	154	15	22*	−	−
68	80	8·5	25·5	−	−	76	85	13	6·5†	−	+
128·1	149·6	9·5	25·5			127·6	139·8	10·5	11·9		

* Appreciable carotene in plasma † Small amounts of carotene in plasma

(iii) *Statistical Evaluation of Analytical Data on Blood.*—Group means, standard errors of means and significant differences for haemoglobin, P.C.V., plasma vitamin A and white cell count are presented in Table 2. There were no significant correlations between plasma vitamin A concentration and either haemoglobin, P.C.V. or white cell count. The final mean plasma vitamin A of the supplemented group was significantly greater than that of the control group ($P < 0{\cdot}01$).

TABLE 2

GROUP MEANS, STANDARD ERRORS OF MEANS AND SIGNIFICANT DIFFERENCES OF SOME CONSTITUENTS IN BLOOD OF HEREFORD CALVES FED SORGHUM GRAIN *ad lib.* WITH AND WITHOUT VITAMIN A SUPPLEMENTATION

Determination	Supplemented		Control	
	Initial	Final	Initial	Final
Blood haemoglobin (g/100 ml)	11·6 ± 0·6	8·9 ± 0·6***	11·8 ± 0·6	10·2 ± 0·6**
P.C.V. (%)	35·8 ± 1·7	34·3 ± 1·6	36·7 ± 1·7	36·7 ± 1·6
Plasma vitamin A (μg/100 ml) ..	9·5 ± 1·5	25·5 ± 2·1***	10·5 ± 1·5	11·9 ± 2·1
White cell count	—	10,025 ± 705	—	8,788 ± 705

** Significant at 1% probability level.

*** Significant at 0·1% probability level.

(iv) *Body-weight.*—A linear growth rate was estimated for each animal, using the weekly body-weights. The mean body-weight gain was 5·5 ± 0·8 and 3·1 ± 0·8 lb per week for the supplemented and control groups respectively. The difference in mean group growth rate did not attain significance, the difference required for significance at 5 per cent. level of probability being 2·6 lb per week.

(v) *Grain Consumption.*—The mean daily grain consumption per head for weekly intervals in each group is presented in Table 3. The feed intake was greater in the supplemented group for all weeks. Further, feed consumption increased every week in the supplemented group, whereas it remained relatively constant in the control group.

TABLE 3

MEAN CONSUMPTION OF SORGHUM GRAIN BY VITAMIN A SUPPLEMENTED AND CONTROL HEREFORD CALVES

Week of Experiment	Mean Grain Consumption (lb/head/day)	
	Supplemented	Control
1	4·98	4·18
2	5·39	4·25
3	5·66	4·22
4	5·93	4·15
MEAN	5·49	4·20

(vi.) *Incidence of Scouring.*—Initially two calves were observed scouring—both had the lowest level of plasma vitamin A (< 4 μg/g) and both were night-blind. One animal was supplemented with vitamin A and stopped scouring within two days; the other was from the control group and scoured intermittently throughout the experiment. No other animals were observed scouring.

V. DISCUSSION

The experimental conditions which led to the collection of this data were the period of six months during which pregnant and lactating cattle were fed on restricted amounts of all-grain rations, and the early weaning of calves on to the same type of ration. Such conditions could be encountered during drought feeding on all-grain rations. From the data obtained in this experiment, it is evident that under these conditions vitamin A supplementation of calves is necessary to prevent clinical effects of vitamin A deficiency. This is true in spite of adequate liver vitamin A reserves in the dams at the commencement of drought feeding.

Guilbert and Hart (1935) established the relationship of nyctalopia and vitamin A deficiency in the bovine. They found that night blindness was the first detectable clinical symptom of the deficiency. Moore (1939*a*, 1939*b*) found that papillary oedema developed at approximately the same time as night blindness in calves, but did not necessarily develop simultaneously.

Some care is necessary in interpreting data relating vitamin A levels in plasma to various physiological conditions in the bovine. Most American workers expressed results in micrograms by multiplying the International Unit by the factor 0·25. Other workers, including ourselves, have used the factor 0·30. Boyer *et al.* (1942) maintained three groups of dairy calves for over a year at blood plasma vitamin A levels of 10-12, 8 and 4 μg/100 ml. Plasma levels of 10-12 μg/100 ml prevented the appearance of papillary oedema as determined by ophthalmoscopic examination over six months. Levels of 7-8 μg/100 ml were borderline and with levels of 4-6 μg/100 ml papillary oedema invariably developed. On the basis of growth rate, Jacobson, Converse, and Moore (1949) considered a calf depleted of vitamin A when the blood plasma contained <4 μg/100 ml for two consecutive weeks. Based on feed consumption and body-weight data, Eaton *et al.* (1951) indicated that the liver reserves of vitamin A and carotene have been essentially depleted in young dairy calves when the blood plasma level reaches <4 μg/100 ml. Eaton *et al.* (1958) considered that when calves were being fed a vitamin A depletion ration, a level of 12 μg/100 ml plasma indicated partial depletion in calves.

Our findings are based on 16 calves with plasma vitamin A levels ranging from <4 to 29 μg/100 ml in which the relationship between nyctalopia and plasma levels was examined on two occasions. Nyctalopia was recorded in nine calves and the plasma levels ranged from <4 to 8 μg/100 ml. Two calves with plasma levels of 8·5 μg/100 ml did not show night blindness and only in one calf was a level of <8 μg/100 ml (7 μg/100 ml) not associated with this condition. It appears from these data that plasma vitamin A levels of 8 μg/100 ml or less are usually associated with nyctalopia in calves. These findings are comparable with those of Wheeler *et al.* (1957). They determined the plasma vitamin A levels in calves about two months after developing night blindness and found a mean value of 4 μg/100 ml (range 1-6 μg/100 ml) in eight affected animals. However, Southcott and McClymont (1960) did not observe night blindness in 38-month-old Hereford steers where nine animals had serum vitamin A levels of 10 μg/100 ml or less (three below 4 μg/100 ml, of which one was 2 μg/100 ml). The level associated with night blindness in sheep is $9 \cdot 4 \pm 4$ μg/100 ml (M.C. Franklin, personal communication).

As these calves had been concerned with an experiment on early weaning and were being used to evaluate the clinical effects of vitamin A supplementation, it was not desirable to undertake liver biopsies to determine their vitamin A reserves. However, the following data indicate that these reserves must have been very low in some animals. Two calves which died two and three weeks before the commencement of the work reported in this paper had liver vitamin A levels of <1 μg/g. The findings of Wheeler *et al.* (1957), Teichman *et al.* (1957) and Rousseau *et al.* (1958) show that plasma vitamin A levels of less than 8 μg/100 ml are associated with liver vitamin A levels of <1 μg/g in the bovine.

The control calves were supplemented orally with vitamin A after the termination of the all-grain feeding period. They were turned out to graze and one week later showed no sign of night blindness. The ability of calves to recover from night blindness within a week of carotene ingestion has also been shown by Moore (1939*a*). Guilbert and Hart (1935) found that the rate of disappearance of night blindness is proportional to the amount of carotene or vitamin A ingested

At the initial sampling, carotene could not be detected in the blood of these calves. This was not unexpected, as the sorghum grain consumed by both dams and calves contained only 0·075 mg beta-carotene per lb (Gartner and Ryley 1962). The presence of carotene and the increase in vitamin A in plasma of some animals in the control group at the final sampling indicate some exposure of these animals to green feed. They either succeeded in eating some grass through the rails of their bare yards or obtained it when being driven from their yards to be weighed. However, this was not observed and the findings of Eveleth, Bolin, and Goldsby (1949) suggest that the exposure may have been exceedingly small. In their work, yearling ewes were accidentally fed some prairie hay after a prolonged period on rations containing negligible amounts of carotenoids. This single supplement of carotene caused a rise in serum vitamin A that did not return to the previous level for nearly 60 days.

In contrast to the general agreement between reports on the effect of vitamin A deficiency on the reticulo-endothelial system, data on associated haematological changes are limited and contradictory. Jungherr, Helmboldt, and Eaton (1950) found no differences in blood haemoglobin levels determined weekly on vitamin A supplemented and depleted bull calves. Frank (1934) reported that in rats one effect of vitamin A deficiency was the reduction in total leucocyte count, whereas in two human infants with xerophthalmia the total white cell count was increased. In our experiment there were no significant differences in blood haemoglobin, P.C.V. and white cell count between supplemented and control calves.

The mean growth rate was higher in the supplemented group than in the control group. This difference approached significance and may have been more marked if some animals in the control group had not had access to some green feed. One animal, initially the third heaviest in the control group, had the lowest plasma vitamin A level at both sampling periods (<4 μg/100 ml) and consistently lost weight between every weekly weighing. Greater liveweight increases in vitamin A supplemented calves compared with vitamin A depleted calves have been shown by Spielman *et al.* (1949). Boyer *et al.* (1942) and Jacobsen, Converse, and Moore (1949) showed further either decreased rate of gain or complete inhibition of growth when plasma vitamin A levels fell to 4 μg/100 ml or less.

The feed consumption data in our experiment demonstrate an increasing mean weekly consumption of the supplemented calves and a relatively constant consumption of the control calves. This would account for the weight differences in favour of the supplemented group. Eaton *et al.* (1951) found that with young dairy calves on a vitamin A depletion ration, feed intake was not affected until blood plasma levels decreased to 4 μg/100 ml. At this stage 6 from a group of 15 calves began to show inappetance.

Scouring has been associated with vitamin A deficiency in calves (Thorp *et al.* 1942; Spielman *et al.* 1949; Jacobson, Converse, and Moore 1949; Eaton *et al.* 1951; Brocklesby 1960). In our experiment only two calves were observed scouring and in these the plasma vitamin A levels were less than 4μg/g. One was supplemented with vitamin A following allocation to the supplemented group and ceased scouring, while the unsupplemented animal continued to scour intermittently.

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