RELATIONSHIP BETWEEN HEPATIC AND BUTTERFAT VITAMIN A CONCENTRATIONS IN BEEF CATTLE RECEIVING NEGLIGIBLE CAROTENE

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SUMMARY

Hereford heifers with a mean stage of gestation of 171 days were transferred from pasture to yards and fed restricted all-grain rations for 24 weeks. The sorghum grain used contained negligible carotene. Liver vitamin A analyses on biopsy samples were done initially and at the beginning of the twenty-fourth week. Butterfat analyses were carried out at the completion of the all-grain feeding period when the mean stage of lactation of the heifers was 70 days.

Mean initial liver vitamin A levels of 300 μg/g (500–149 μg/g) declined to 150 μg/g (354–54 μg/g).

The vitamin A potency of the butterfat averaged 24·2 I.U./g (10·2–39·4 I.U./g). The butterfat did not contain detectable carotenoids. The concentration of vitamin A in butterfat declined as yield increased.

Vitamin A in butterfat was significantly correlated with vitamin A in liver (r = + 0·71).

Final levels of liver vitamin A were significantly correlated with initial levels (r = + 0·80).

In this method of feeding, adequate initial reserves of vitamin A are necessary in the dams to ensure the immediate vitamin A requirement of the suckling calf.

I. INTRODUCTION

The use of restricted amounts of crushed sorghum grain has been studied experimentally with a view to feeding cattle for survival during drought (Morris 1958; Ryley, Gartner, and Morris 1960; Ryley and Gartner 1962). In common with most cereal grains sorghum contains little carotenoids, and when fed in restricted quantities as the sole ration the amount of carotene supplied to the animals is negligible. The effects of carotene depletion under simulated drought feeding conditions were therefore examined in beef cattle fed restricted amounts of crushed sorghum grain as the sole diet for periods of approximately six months.

Studies on the depletion of liver vitamin A concentration in maiden Hereford heifers were included in the paper by Ryley, Gartner, and Morris (1960). Four groups of 10 heifers were fed at the rate of 3 lb of crushed sorghum grain per

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head per day for 26 weeks. The mean liver vitamin A declined in all groups from 157 to 84 μg/g and of the 20 animals selected for sampling one had a low (1·5 μg/g) and two others a marginal (5 and 10 μg/g) liver vitamin A reserve at the end of the experiment. However, there was no clinical evidence of night blindness. The authors concluded that the need for vitamin A supplementation would be related to the class of cattle, initial reserves, and the duration of feeding rations containing negligible amounts of carotenoids.

Studies on pregnant and lactating cattle fed for survival on sorghum grain alone under simulated drought feeding conditions have been reported by Ryley and Gartner (1962). Hereford heifers, pregnant for a mean period of 171 days, were carried through a feeding period of 24 weeks. The opportunity was taken to examine the following relationships: (*a*) liver and butterfat vitamin A concentrations, (*b*) initial to final vitamin A concentrations, and (*c*) butterfat vitamin A concentration and yield, all under conditions of negligible carotene intake. The results 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). A covered creep measuring 8 ft square was provided in each of the three yards.

(ii) *Experimental Animals.*—The experimental animals in this study were 20 dehorned, pregnant Hereford heifers previously used as maiden animals in a drought feeding experiment (Ryley, Gartner, and Morris 1960). Subsequently they had been grazing *Paspalum dilatatum* pastures at the Animal Husbandry Research Farm, Rocklea, for 16 months. They were then used in the experiment on the feeding of restricted amounts of crushed sorghum grain to cattle in late pregnancy and early lactation (Ryley and Gartner 1962). At the commencement of this experiment their mean age was approximately 40 months and their mean stage of gestation was 171 days.

(iii) *Sorghum Grain.*—A single consignment of *Sorghum vulgare* L. grain (Alpha variety) was purchased prior to the commencement of the experiment and crushed prior to feeding.

(iv) *Sampling and Analysis of Grain.*—A 1 lb sample was obtained from each 200 lb of grain just prior to mixing with 1 per cent. ground limestone. The samples were retained in a closed container and at the conclusion of the drought feeding experiment were mixed and subsampled for analysis. The method of estimation of beta-carotene was that used by Hoffmann–La Roche.

(v) *Determination of Milk Production.*—All heifers were segregated from their calves and hand-milked on the last day of the drought feeding experiment. They were milked again, in the same order, 4 hr later, and again 24 hr after the second milking. An intravenous injection of 10 I.U. oxytocin in 10 ml of physiological saline was given to each cow during milking. The time of commencement and completion of each milking was recorded for each animal and the mean used to calculate 4-hr and 24-hr milk yield. The 24-hr milk yield is used in this paper. The weight of milk was obtained on a physical balance with an accuracy of $\pm$ 1 g.

(vi) *Separation of Fat from Milk*.—Approximately two pints of milk were obtained from the 24-hr milk yield of each animal. The milk was chilled overnight, causing the cream to separate as a distinct layer. The bulk of the milk was removed from under the cream layer by suction and the cream shaken until dispersal of the fat granules and separation of the buttermilk occurred. The buttermilk was decanted, the granules melted over a boiling water-bath, centrifuged and the fat decanted into containers. These were stored at approximately 4°C until analysed.

(vii) *Methods of Chemical Analysis*.—Analytical methods employed were as follows:—milk fat, Burgess (1936); butterfat vitamin A, essentially that of McDowell (1949) as outlined by Gartner (1959). Samples of liver were obtained by the aspiration biopsy technique of Loosemore and Allcroft (1951), using the instruments described by Dick (1952), and the vitamin A concentration analysed as described by Ryley, Gartner, and Morris (1960).

III. EXPERIMENTAL

Pregnant heifers with a mean stage of gestation of 171 days were removed from pasture, allotted to three groups, transferred to yards in their respective groups, and changed to an all-grain ration over a period of two weeks. They were maintained on grain for a further period of 24 weeks, calving taking place between the eleventh and fourteenth week of grain feeding. Heifers were removed from the experiment if their calves were born dead or died during the experimental period. This ensured that all animals in the experiment were either pregnant or suckling their calves. All heifers were segregated from their calves and hand-milked at the conclusion of the experiment, when their mean stage of lactation was 70 days (range 56–85 days). The group numbers at this stage and their respective treatments throughout the experiment were as follows:—

Group	No. of Animals	Crushed Sorghum Grain* (lb/head/day)	Frequency of Feeding
I	5	6	Daily
II	8	6	Twice-weekly
III	7	10	Daily

* To the sorghum grain was added 1 per cent. ground limestone.

The feed was distributed evenly along the feed trough. In each yard the length of troughing allowed approximately 3 ft per animal. Animals were fed between 9 a.m. and 10 a.m. Group II received 18 lb per head each Tuesday morning and 24 lb per head each Friday morning.

All calves were allowed unrestricted access to a creep containing crushed sorghum grain with 1 per cent. added limestone, after the youngest calf in the experiment reached four weeks of age. They were weaned four weeks later when their dams had completed the 24 weeks' period of all-grain feeding. The average age of the calves at weaning was 10 weeks (range 56–85 days).

Liver biopsy samples for vitamin A analyses were obtained from the heifers on the day the animals were transferred from pasture to the drought feeding yards and again at the beginning of the twenty-fourth week of all-grain feeding.

IV. RESULTS

(a) Heifers

Of the 28 pregnant heifers in the experiment at the beginning of the grain feeding period, 20 were suckling their calves at the end of 24 weeks. This decrease in numbers was due to the following causes:—one heifer aborted after the first two weeks, one died after giving premature birth to twins, one died following prolonged calving, one was withdrawn after 22 weeks following an accident, and three were withdrawn after their calves had died. Liver samples were also collected from these eight heifers which had failed to meet the requirements of the drought feeding experiment. Their liver vitamin A levels when they were removed from the experiment ranged from 59 to 284 μg/g.

(b) Analysis of Grain

The sorghum grain consumed by the heifers and their calves contained 0·075 mg beta-carotene per lb.

(c) Milk Yield, Butterfat Content, Liver and Butterfat Vitamin A Concentration

The individual results and group means for 24-hr milk yield, butterfat percentage, 24-hr butterfat yield, total vitamin A potency of butterfat, 24-hr secretion of butterfat and liver vitamin A concentrations at the commencement of all-grain feeding and 24 weeks later are recorded in Table 1. The liver biopsy samples were obtained a week before the 24-hr milk samples. The butterfat secreted by all the animals was colourless and did not contain detectable carotenoids. The amount of milk secreted was related to the amount of sorghum grain fed, Group III, receiving 10 lb per head per day, having a higher average milk yield than either Groups I or II, fed at the rate of 6 lb per head per day.

TABLE 1

LIVER VITAMIN A CONCENTRATIONS, 24-HR MILK YIELD, BUTTERFAT PERCENTAGE, 24-HR BUTTERFAT YIELD, TOTAL VITAMIN A POTENCY OF BUTTERFAT, AND 24-HR SECRETION OF BUTTERFAT IN HEREFORDS FED RESTRICTED AMOUNTS OF SORGHUM GRAIN

Group	Liver Vitamin A (μg/g)		24-hr Milk Yield	Butterfat	24-hr Butterfat Yield	Butterfat Vitamin A	24-hr Vitamin A Secretion
	*From Pasture 3.v.60	Final Grain 26.x.60	(g)	(%)	(g)	(I.U./g)	(I.U.)
I	395	85	1,735	4·90	85·0	13·9	1,182
	351	245	1,282	6·00	77·0	23·8	1,833
	383	238	320	3·10	9·9	22·8	226
	184	58	1,805	3·30	59·6	11·5	685
	220	97	230	8·80	20·2	24·5	495
MEAN ..	307	145	1,074	5·20	50·3	19·3	884
II	503	319	839	3·00	25·2	37·1	935
	314	122	1,565	3·65	57·1	29·1	1,662
	173	75	1,201	3·40	40·8	25·2	1,028
	282	190	597	4·05	24·2	39·4	954
	236	176	1,090	3·05	33·2	36·3	1,205
	243	116	1,050	3·20	33·6	38·0	1,277
	149	59	708	3·35	23·7	27·1	642
	177	54	545	3·25	17·7	36·0	637
MEAN ..	260	139	949	3·37	31·9	33·5	1,043
III	262	64	2,011	4·35	87·5	14·5	1,269
	412	354	855	5·35	45·8	28·1	1,287
	319	149	1,774	2·00	35·5	20·3	721
	234	103	2,575	5·70	146·8	12·5	1,835
	298	166	2,607	3·70	96·5	17·7	1,708
	305	81	2,155	3·10	66·8	10·2	681
	394	228	3,290	5·30	174·3	16·4	2,859
MEAN ..	318	164	2,181	4·21	93·3	17·1	1,480

* Determinations listed in this column were carried out two weeks prior to all-grain feeding; all other values in this table were obtained after 24 weeks of all-grain feeding.

The correlation coefficients have been calculated on a within-group basis and are presented in Table 2. Each coefficient is based on 16 degrees of freedom.

These data show:—

(1) That the concentration of butterfat vitamin A was positively related to liver concentration at the conclusion of grain feeding (October 26, 1960) and also tended to be related to the initial concentrations from pasture (May 3, 1960).

(2) That the liver vitamin A level of all animals fell during the experimental period, but animals preserved their ranking—i.e. those with highest initial levels also had the highest final levels.

(3) That animals with high milk yields were also high producers of butterfat and vitamin A. However, the concentration of vitamin A in butterfat declined as yields increased.

In addition, the correlation between 24-hr secretion of vitamin A and percentage decline in liver vitamin A (May 3, 1960–October 26, 1960) was examined. The correlation coefficient (r = — 0·24) was not significant at the 5 per cent. level.

TABLE 2

CORRELATION COEFFICIENTS BETWEEN LIVER VITAMIN A CONCENTRATIONS, 24-HR MILK YIELD, BUTTERFAT PERCENTAGE, 24-HR BUTTERFAT YIELD, TOTAL VITAMIN A POTENCY OF BUTTERFAT, AND 24-HR SECRETION OF BUTTERFAT IN HEREFORDS FED RESTRICTED AMOUNTS OF SORGHUM GRAIN

Determinations	Correlation Coefficient					
	Liver Vitamin A		Butterfat	24-hr Butterfat Yield	Butterfat Vitamin A Concentration	24-hr Vitamin A Secretion
	3.v.60	26.x.60				
24-hr milk yield	−0·05	−0·30	−0·13	+0·85	−0·63	+0·68
Butterfat	−0·08	+0·07		+0·29	+0·26	+0·39
24-hr butterfat yield ..	+0·02	−0·12			−0·42	+0·87
Butterfat vitamin A concentration	+0·42	+0·71				−0·03
24-hr vitamin A secretion	+0·24	+0·23				
Liver vitamin A 26.x.60 ..	+0·80					

The significant levels are:—

5% : coefficient exceeding 0·47.

1% : coefficient exceeding 0·59.

0·1% : coefficient exceeding 0·71.

V. DISCUSSION

The sorghum grain consumed by the heifers contained 0·075 mg beta-carotene per lb. This, fed at the higher rate of 10 lb per day, provided 0·75 mg beta-carotene per animal per day. Body-weight of the heifers during the last three months of gestation averaged 820 lb and during the first three months of lactation 672 lb (Ryley and Gartner 1962). Thus during gestation and lactation they received a daily average intake of 0·09 and 0·11 mg beta-carotene per 100 lb body-weight respectively. This is well below requirements. Wheeler *et al.* (1957) indicated that a daily carotene intake of 1·5 mg per 100 lb liveweight in animals with some hepatic vitamin A storage was adequate for gestation and parturition. The initial vitamin A levels of their experimental cows (estimated from tables, graphs and liver dry-matter content) was 50–60 μg/g. Page *et al.* (1958) indicated that an average daily intake of 7·54 mg carotene per 100 lb body-weight was required to maintain initial hepatic vitamin A content in steers. The National Research Council (1958) recommendations stated that beef cows had a daily requirement of 3–4 mg carotene per 100 lb body-weight during pregnancy and 10 mg per 100 lb body-weight during lactation in order to produce strong calves at birth and to supply the vitamin A needs of suckling calves during the first 3–4 months of age.

The results of our experiment have indicated that when the initial liver vitamin A reserves of Hereford heifers are of the order found (300 μg/g), they can 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 while receiving only negligible amounts of carotenoids. When the calves were weaned, the liver vitamin A reserves of all their dams averaged 150 μg/g, and all levels, including the minimum level of 54 μg/g, would be considered adequate.

In a previous drought feeding experiment (Ryley, Gartner, and Morris 1960) a representative number of the same animals used in this experiment, then maiden heifers, had average initial liver vitamin A reserves of 157 μg/g. After a comparable period of approximately six months on an all-grain ration, likewise containing negligible carotene, the levels of vitamin A in liver decreased to a mean of 84 μg/g. However, one had a low (1·5 μg/g) and two others a marginal (5 and 10 μg/g) liver vitamin A concentration.

From both experiments it is evident that the greater the initial vitamin A reserve in liver the less the chance that this reserve will be depleted completely during periods of up to six months on rations containing virtually no carotene. This is true even with the additional requirements imposed by lactation. This supports the hypothesis that the rate of depletion of the body reserves of a vitamin are directly proportional to the total reserves of that particular vitamin in the body (Hickman, 1946; Frey and Jensen, 1947).

In cattle, vitamin A is transported from the intestines to the storage organs mainly in the esterified form (Kon, McGillivray, and Thompson 1955). This vitamin A, together with unchanged carotene, is associated during transport from the intestine to the storage organs with dietary fat in the chylomicrons. In lactating cattle it is the uptake of these by the mammary gland which would account for the bulk of the carotene and vitamin A in the milk fat (McGillivray 1957). When cows are deprived of carotene, vitamin A is mobilized from the liver and secreted into the milk fat (Chanda and Owen 1952; Chanda, Clapham, and Owen 1954). In our experiment virtually only liver reserves of vitamin A were available. There is therefore a sound basis for evaluating the relationship of vitamin A in liver to vitamin A in butterfat in these animals.

Because of the highly significant relationship between vitamin A concentrations in liver and butterfat ($r = +0·71$, $P = 0·001$), under conditions of low carotene intake, one would expect low concentrations of vitamin A in butterfat from animals with low liver vitamin A concentrations. As part of a study of carotene metabolism in beef cattle, Baker *et al.* (1953) reported the effects of maternal liver stores of vitamin A and level of carotene intake on the vitamin A content of the milk of cattle after three months' lactation. Two cows receiving a low carotene ration for the last six months of gestation and the first three months of lactation had a mean liver vitamin A level at the end of this period of 0·6 μg/g associated with a mean butterfat vitamin A concentration of only 2·8 I.U./g (recalculated from liver dry-matter basis and assuming the milk analysed contained 4 per cent. butterfat). Subsequently Baker, Pope, and MacVicar (1954) repeated this work to include cows with higher initial liver stores of vitamin A. However, the mean liver vitamin A level of five cows three months after parturition was only about 10 μg/g and the concentration of vitamin A secreted in the butterfat at this time was of the order of 3·8 I.U./g (same basis of recalculation as above). Majumdar and Gupta (1960) did not detect vitamin A in either the milk or liver of cows maintained over a period of more than 1½ years on a vitamin A deficient regimen. These data indicate that under conditions of low carotene intake the positive relationship of liver and butterfat vitamin A is not only apparent at normal levels of liver vitamin A, but also at levels below that found in our animals.

The liver vitamin A concentration of all animals fell during the experimental period, but animals preserved their ranking—i.e. those with the highest initial levels also had the highest final levels. This is in agreement with the findings of Page *et al.* (1958) and Ryley, Gartner, and Morris (1960).

As vitamin A in butterfat is positively correlated with vitamin A in liver ($r = +0 \cdot 71$; $P = 0 \cdot 001$) and as initial and final levels of liver vitamin A are related ($r = +0 \cdot 80$; $P < 0 \cdot 001$), it is evident that in this method of drought feeding adequate initial levels of vitamin A are necessary in the dams to ensure the immediate vitamin A requirement of the suckling calf. It is doubtful whether animals in Queensland will enter a period of drought feeding with the high reserves encountered in this experiment. The animals would usually have been on dry feed previous to any supplementary feeding and their liver vitamin A reserves may be considerably lower. In this case it could be desirable to supply the dam with this vitamin during gestation and lactation. On the other hand, if it is thought the initial vitamin A status of the dam is adequate and the consideration is to ensure the supply of this vitamin to the calf, then it would be more efficient to supply vitamin A direct to the calf rather than through the dam.

The concentration of vitamin A in butterfat declined as yield increased. This decline was pronounced in data from a survey of Queensland butterfats where marked seasonal trends occurred in carotene, vitamin A and total vitamin A contents (Gartner 1959). In this survey there was a highly significant negative correlation ($r = -0 \cdot 75$; $P < 0 \cdot 01$) between production ratio and the total vitamin A potency of butterfats, production ratio being the ratio of percentage butter produced in any one month to percentage cows lactating in the same month. It was concluded that the influence of production ratio may account for some of the variations in butterfat potencies throughout the year or part of the year.

The vitamin A potency of the butterfat secreted by the heifers was variable, ranging from 10·2 to 39·4 I.U./g, with a mean of 24·2 I.U./g. This is considerably less than the minimum of 42·6 I.U./g found by Gartner (1959) for dairy cattle in south-eastern Queensland. The fact that there was virtually no carotene ingested for 24 weeks by the beef cattle in this experiment would explain this difference.

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REFERENCES

BAKER, F. H., MACVICAR, R., POPE, L. S., and WHITEHAIR, C. K. (1953).—Placental and mammary transfer of vitamin A and carotene by beef cows. *Proc. Soc. Exp. Biol., N.Y.* 83:571-4.

BAKER, F. H., POPE, L. S., and MACVICAR, R. (1954).—The effect of vitamin A stores and carotene intake of beef cows on the vitamin A content of the liver and plasma of their calves. *J. Anim. Sci.* 13:802-7.

BURGESS, L. A. (1936).—The Babcock test. *Qd Agric. J.* 46:633-45.

CHANDA, R., and OWEN, E. C. (1952).—The effect of thyroxine and thiouracil on the composition of milk. 2. The carotene and vitamin A content. *Biochem. J.* 51:404-9.

CHANDA, R., CLAPHAM, HELEN M., and OWEN, E. C. (1954).—Dietary carotene and the degree of esterification of vitamin A in the milk and blood of cows. *Biochem. J.* 56:453-8.

DICK, A. T. (1952).—Improved apparatus for aspiration biopsy of the liver in sheep. *Aust. Vet. J.* 28:234-5.

FREY, P. R., and JENSEN, R. (1947).—Depletion of vitamin A reserves in the livers of cattle. *Science.* 105:313.

GARTNER, R. J. W. (1959).—Variations in the carotene and vitamin A contents of Queensland butterfats. *Qd J. Agric. Sci.* 16:1-30.

HICKMAN, K. C. D. (1946).—*Interne*: 278 (Quoted by Frey and Jensen 1947).

HOFFMAN—LA ROCHE.—"Determination of Beta-carotene in Food and Feedstuffs". (Basle, Switzerland).

KON, S. K., MCGILLIVRAY, W. A., and THOMPSON, S. Y. (1955).—Metabolism of carotene and vitamin A given by mouth or vein in oily solution or aqueous dispersion to calves, rabbits and rats. *Brit. J. Nutr.* 9:244-67.

LOOSEMOORE, R. M., and ALLCROFT, RUTH (1951).—Technique and use of liver biopsy in cattle. *Vet. Rec.* 63:414-6.

MCDOWELL, A. K. R. (1949).—The estimation of vitamin A in butter. A critical study of methods. *J. Dairy Res.* 16:348-55.

MCGILLIVRAY, W. A. (1957).—Factors influencing the vitamin A content of milk fat. The source of milk fat vitamin A and carotene. *J. Dairy Res.* 24:102-7.

MAJUMDAR, B. N., and GUPTA, B. N. (1960).—Vitamin A deficiency survey in cattle at Nagaur and Bikaner (Rajasthan). *Ann. Biochem.* 20:273-8.

MORRIS, J. G. (1958).—Drought feeding studies with cattle and sheep. 3. A preliminary note on the use of grain sorghum as a drought fodder for cattle. *Qd J. Agric. Sci.* 15:195-202.

NATIONAL RESEARCH COUNCIL (1958).—Recommended nutrient allowances for domestic animals. IV. Recommended nutrient allowances for beef cattle.

PAGE, H. M., ERWIN, E. S., VARNELL, T. R., and ROUBICEK, C. B. (1958).—Effect of hepatic vitamin A and carotene concentration on the biological value of carotene in the bovine. *Amer. J. Physiol.* 194:313-5.

RYLEY, J. W., GARTNER, R. J. W., and MORRIS, J. G. (1960).—Drought feeding studies with cattle. 5. The use of sorghum grain as a drought fodder for non-pregnant heifers. *Qd J. Agric. Sci.* 17:339-59.

RYLEY, J. W., and GARTNER, R. J. W. (1962).—Drought feeding studies with cattle. 7. The use of sorghum grain as a drought fodder for cattle in late pregnancy and early lactation. *Qd J. Agric. Sci.* 19:309-30.

WHEELER, R. R., WESWIG, P. H., BRANNON, W. F., HUBBERT, F. E. Jr., and SAWYER, W. A. (1957).—The carotene and vitamin A content of plasma and liver of range Hereford cows and their calves in the Northern Great Basin. *J. Anim. Sci.* 16:525-36.

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