INFLUENCE OF PROTEIN DEPLETION ON VITAMIN A AND CAROTENE
UTILIZATION BY VITAMIN A DEFICIENT SHEEP.

by

Thomas A. Anderson

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SIGNED: Thomas A. Anderson

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Farris Hubbert, Jr.
Farris Hubbert, Jr.

Associate Professor of Animal Science

12-6-60
Date

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INTRODUCTION

As the wealth of research on vitamin A accumulates it is becoming increasingly evident that delicate physiological and biochemical interrelationships affect its metabolism. The various factors influencing vitamin A metabolism make it impossible to establish an exact minimum daily requirement per se. The daily vitamin A needs of a particular animal are known to be influenced by such factors as age, sex, composition of the diet, stage of production, ambient temperature and humidity, state of health, plane of nutrition, and basal metabolic rate.

An area of vitamin A research left largely unexplored is the relationship of the level of dietary protein to vitamin A and carotene utilization. Some work with monogastric animals appears in the literature but information on this subject using ruminant animals is extremely scarce.

The question is certainly one of practical importance since, under range conditions, periods of low protein intake are usually coincident with periods of low carotene content in the forage. A significant interrelationship between level of dietary protein and vitamin A and carotene utilization might suggest a second look at the accepted minimum daily requirements of vitamin A for sheep during the dry grass season.

This research was conducted to determine the influence of dietary

protein deficiency upon total serum protein and serum protein fractions of sheep. The influence of protein deficiency on carotene conversion to vitamin A and upon vitamin A utilization and storage was also studied. A final objective of this experiment was to determine what effect vitamin A and carotene administration have upon total serum protein and serum protein fractions.

REVIEW OF LITERATURE

No evidence of vitamin A deficiency occurring spontaneously in sheep was found in the literature. Vitamin A deficiency has been produced experimentally in sheep, although much less readily than in the bovine species. The resistance of sheep to vitamin A deficiency suggests an efficient absorption and conversion of carotene to vitamin A. Evidence of this efficiency is plain in noting the lack of circulating carotene in the blood plasma and the white body fat. Plasma carotene levels in the bovine are normally higher than plasma vitamin A levels and the body fat is yellow, particularly when grazing green pasture. These differences in carotene concentration within the body imply that the ovine is more efficient than the bovine in the conversion of carotene to vitamin A.

Normal plasma levels of vitamin A for sheep have been reported as 37-48 mcg. % by Pope et al. (42), 25-35 mcg. % by Peirce (40), 22-48 mcg. % by Wisconsin researchers (50) and 20-45 mcg. % by Hoefer and Gallup (23). Plasma carotene levels are usually not measurable although Peirce (40) reported levels of 5-15 mcg. % when sheep were grazing green pastures.

Normal average hepatic vitamin A reserves for sheep have been estimated to be 503 I. U. per G. of liver by Harms (21), 460 I. U. per g. by Moore and Payne (33) and 600 I. U. per g. by Moore (32). Peirce

(40) in his investigations with Merino sheep found liver vitamin A stores to vary from 230-810 I. U. per g.

In 1940 Guilbert et al (19) observed that the daily intake of vitamin A per kg. body weight required to prevent night blindness in sheep was 17-26 I. U. (4.3-6.3 mcg.) and that of carotene 42-58 I. U. (25-35 mcg.). The National Research Council (36) has adopted these values, setting 17 I. U. of vitamin A or 25 mcg. of carotene per kg. body weight as the minimum daily requirements for sheep. It is emphasized, however, that approximately 3 times this amount is needed for liver storage and about 5 times this minimum amount for pregnancy and lactation.

Guilbert, Miller and Hughes (20) found that it took 22-23 months on a vitamin A deficient ration to produce symptoms of vitamin A deficiency in a group of 7-year old ewes. These ewes had initial hepatic vitamin A stores of 625-1000 I. U. per g. of liver. After 10-16 months the reserves had fallen to 50-150 I. U. per g. and at 27-30 months no liver vitamin A was demonstrated. Withdrawal of hepatic vitamin A reserves was more rapid in the first year than it was in the second, indicating a less efficient use of high reserves.

In Australia, Peirce (39) demonstrated that a daily carotene intake of only 10 mcg. per kg. body weight permitted satisfactory growth in lambs for one year, after which time continuation on the diet led to night blindness, low plasma and liver vitamin A levels, muscular incoordination and, finally, death. Initial liver vitamin A concentrations of 200 mcg. per g. of liver fell to 100 mcg. per g. in

2 months, to 35 mcg. per g. in 4 months and to 4 mcg. per g. in 9 months. The plasma vitamin A level remained at the initial level of 35 mcg. % for 3 months, reached 23 mcg. % at 6 months, 13 mcg. % at 9 months and a constant 6 mcg. % level from the thirteenth month. These data support the earlier findings of Guilbert et al. (19) with sheep and later research by High (22) with rats that higher liver vitamin A reserves are used less efficiently than lower stores. The plasma level of vitamin A is usually maintained within the normal range until the liver reserves are insufficient to continue this level. The blood level falls more slowly as depletion increases. Supplementation of dried lucerne leaves providing 25-30 I. U. of carotene per kg. body weight was effective in arresting the fall in the plasma vitamin A level of sheep which had been on the experiment for a period of 9 months. This level of dosing did not cure night blindness or provide any liver stores of vitamin A. Doses of 50-55 I. U. of carotene per kg. body weight were effective in curing night blindness but had no effect upon liver stores.

Hoefer and Gallup (23) state that no simple relationship seems to exist between the level of plasma vitamin A of fattening lambs and liver vitamin A stores when the plasma level was above 20 mcg. %. Work by Frey et al. (16) supports the finding that there is no relationship between liver storage of vitamin A in cattle and plasma levels of the vitamin.

Experiments with vitamin A deficient calves by Rousseau et al. (45) showed that plasma vitamin A concentrations expressed as logarithms

were directly related to liver vitamin A concentrations expressed as logarithms, but the upper and lower limits of the plasma vitamin A concentration were placed at 25.8 and 3.6 mcg. % respectively.

Myers et al. (35) found that the plasma vitamin A levels in lambs and pigs increased at a diminishing rate while liver vitamin A concentrations increased at essentially constant rates with increasing intakes of carotene or vitamin A. This pointed out a linear relationship between plasma vitamin A and the logarithm of carotene intake which supports the findings of Repp and Watkins (44). The linear relationship between the logarithm of liver vitamin A and the logarithm of carotene intake is in agreement with work by Frape et al. (15) investigating the vitamin A requirement of the young pig.

The effect of level of dietary protein on the blood constituents in sheep is not too clear. Whiting et al. (49) state that the total serum protein and/or serum albumin content of the blood were not very sensitive indices of the protein requirement of the mature ewe. No significant differences in the level of serum protein were noted until late in the lactation period when ewes on the lowest protein level showed significantly lower serum protein and albumin. The levels of protein fed were 7.5, 10.4 and 12.6 % for a one year period.

However, Klosterman et al. (27) found that pregnant ewes fed at the level of 0.18 lb. of digestible protein per ewe per day had significantly lower total serum protein and serum albumin than those fed at the level of 0.21 or 0.33 lb. of digestible protein per day. Serum globulins were unaffected by level of protein in the diet. Similar findings were made by Dalgarno et al. (10) who reported that

pregnant ewes fed 0.11-0.08 lb. protein equivalent per day had significantly lower serum protein levels (4.36 g. %) than ewes fed 0.19-0.25 lb. protein equivalent per day (5.07 g. %). The same relationships existed when the serum proteins of the fetuses taken 10 days before parturition and the new born lambs were tested and compared to their dams.

Studies of the effect of protein depletion on dogs (1) have indicated similar drops in total serum proteins as well as a decrease in plasma volume. Electrophoretic fractionation of blood plasma taken from protein depleted dogs has shown striking decreases in serum albumin. Zeldis et al. (51) found that the decrease in canine plasma albumin was accompanied by only slight decreases in total globulins and in some instances a slight rise in the globulin fraction. On protein repletion the tissue proteins and the globulin fractions were replenished first and the albumin later. This would suggest that plasma globulins and tissue proteins enjoy prior demands on the total available pool of body proteins in emergency.

Chow et al. (8) also working with protein depleted dogs noted a drop in plasma albumin but this was accompanied by a similar drop in gamma globulin. The other globulin fractions remained unchanged. Protein repletion resulted in an increase in globulin and albumin with the albumin fraction continuing to increase after the globulin fraction reached the normal level.

Lower plasma albumin levels in protein depleted baby pigs were observed by Peo et al. (41) while the globulin fraction remained essentially unchanged. The albumin-globulin ratio decreased with

protein depletion and increased to the normal level upon repletion. Total serum protein was little affected by protein depletion but the hematocrit values were definitely lower in the protein depleted pigs. It was noted that changes in serum protein levels can be masked by changes in blood volume.

A study of the effect of protein depletion and inanition upon rats was described by Weimer and Nishihara (48) where the albumin g. % value of the serum remained unchanged. Significant decreases in serum globulin g. % and in total serum protein g. % were found in both the starved rats and those receiving protein-free diets. There were significant increases in both hematocrit values and in the albumin-globulin ratio in the treatment groups when compared with the controls. These findings are the reverse of those of Peo et al. (41) in their study with the baby pig.

Vitamin A deficiency has been shown to affect the serum protein patterns in both ruminants and in monogastric animals. Erwin, Elam and Dyer (13) demonstrated with vitamin A deficient steers that serum albumin was increased 5.6%, alpha globulin reduced 3% and beta globulin reduced 5.8% when oral dosages of 15 mg. of carotene per lb. body weight were given for 10 days. Liver vitamin A stores dropped in the vitamin A deficient steers but rose from 23 mcg. per g. to 38 mcg. per g. in the normal steers suggesting an impaired conversion of carotene in vitamin A deficiency. Total serum protein was not affected by vitamin A deficiency or by the administration of carotene. Further work by Erwin et al. (14) using identical twin heifers supported the earlier

research. When one twin was subjected to vitamin A deficiency serum albumin was progressively reduced. Intravenous injections of carotene were ineffective in raising the serum albumin content of the blood. This apparent inability of the bovine to convert metabolic carotene to vitamin A was in agreement with the findings of Church et al. (9) and Kon et al. (28). Ten days after an intravenous injection of vitamin A acetate the serum albumin level was raised to normal.

Vitamin A deficiency in the rat produced significantly lower serum albumin levels and significantly higher globulin levels in a study by Cama et al. (7). Similar results were obtained by Pavel et al. (38) who subjected rats to a vitamin A deficient diet for 65 days. In addition to the usual deficiency symptoms a 10% decrease in total protein, 44% increase in serum globulin and a 34% decrease in serum albumin were noted compared to the controls.

An impaired utilization of carotene in vitamin A deficient ruminants has been previously mentioned in the work of Erwin et al. (13). Diven and Erwin (11) reported that vitamin A deficient sheep stored 9.1 times less liver vitamin A than the control animals. Increased carotene intake was necessary to obtain desired responses in cows when initial concentrations of vitamin A were low as shown in the work of Byers et al. (6). Administration of up to 1,250,000 I. U. of vitamin A once every 5 days for 25 days resulted in only a slight increase in plasma and liver vitamin A levels in vitamin A deficient cows.

Literature on the dietary protein-vitamin A interrelationship in ruminants has not been found. Studies with rats have given conflict-

ing results with some researchers (2), (29) finding that protein deficient rats stored less liver vitamin A while others working with chicks (37) and rats on both energy deficient (24) and protein deficient diets (43) found more hepatic vitamin A storage in the depleted animals.

Mayer and Krehl (30) noted that protein deficiency increased the survival time of vitamin A deficient rats. The higher protein diets increased the severity of vitamin A deficiency symptoms. In this same area of study McLaren (31) observed that protein deficiency did not hasten the onset of xerophthalmia in rats and that severe protein deficiency actually delayed the onset of eye symptoms.

The antagonism of high protein diets to vitamin A liver reserves seems to be due to the more rapid growth of young animals on a higher plane of nutrition. Faster growth possibly requires greater amounts of vitamin A to be metabolized, hence, less chance for the accumulation of liver stores.

Viewing this interrelationship from another approach Brown and Morgan (5) observed that the catabolic processes of protein metabolism were abnormally stimulated in the vitamin A deficient rat. Young vitamin A deficient rats only utilized protein 55-62% as efficiently as the normal animals. The young rats on the high protein diet (22% casein) receiving vitamin A retained 38% of their nitrogen intake as compared with a 45% nitrogen retention in the vitamin A supplemented rats on the low protein diet (18% casein). This 7% difference in nitrogen retention between the two groups indicates a less efficient use of nitrogen by the rats on the high protein diet since this group received 40% more dietary protein than similarly treated rats on the low protein diet.

However, vitamin A deficient rats on the high protein ration retained 27% of their nitrogen intake whereas the rats on the vitamin A deficient-low protein diet retained only 16% of the nitrogen intake. Adult rats exhibited no differences in nitrogen metabolism suggesting that vitamin A is more essential for growth of tissue protein than for maintenance.

In support of this work Gebhardt (18) also found that vitamin A had a pronounced influence upon nitrogen balance in rats. This was particularly true in the case of endogenous nitrogen. Studies were conducted which showed that the minimum level of carotene to support optimal nitrogen metabolism was 5-10 mcg. per 100 g. body weight.

EXPERIMENTAL PROCEDURE

The 36 sheep used in the experiment were selected from a group of mature ewes and wethers of mixed breeding that had been on a carotene deficient ration for over 200 days. Twenty-seven ewes and 9 wethers were randomly allotted to one of 6 treatment groups. Care was taken to equalize the number of wethers in each group in order to minimize the influence which sex might have upon the data from any one experimental group (4, 25, 32).

Phase I. The experiment was conducted in 2 phases. The first period of 50 days (April 19, 1960 - June 7, 1960) consisted of a preliminary protein depletion phase during which 3 treatment groups received a protein adequate ration (15.2% crude protein) and the other 3 groups were fed a protein deficient ration (5.5% crude protein). The composition of the 2 essentially carotene-free rations was:

	<u>Protein Adequate</u>	<u>Protein Deficient</u>
Bermuda straw	1430	1670
Milo	300	300
Cottonseed meal	220	-
Urea	20	-
Salt	20	20
Bone meal	<u>10</u>	<u>10</u>
	2000	2000

Blood samples were collected initially and at approximate 14-day intervals from all animals during this period. Symptoms of protein deficiency were verified by electrophoretic fractionation of blood

serum and the measurement of total serum protein, hematocrit and weight loss. At the end of the first phase all sheep were bled by venous puncture and liver samples were taken by a modification of the aspiration biopsy technique reported by Erwin et al. (12).

Phase II. During the second period of 28 days (June 7, 1960 - July 5, 1960) the protein deficient group was further depleted while both the protein adequate and protein deficient groups received daily oral doses of vitamin A acetate and beta carotene. Within each of the 2 protein levels one group of 6 sheep served as the control, another group of 6 animals received 25 mcg. vitamin A acetate per kg. body weight, and the third group of 6 animals received 25 mcg. beta carotene per kg. body weight. Table 1 shows the number of animals involved in each treatment observation during the experiment.

Table 1. Number of Animals Used Within Each Treatment						
Date	15.2% Crude Protein Ration			5.5% Crude Protein Ration		
	Control	Oral Carotene	Oral Vitamin A	Control	Oral Carotene	Oral Vitamin A
			(Initial)			
4/19	6	6	6	6	6	6
			(End First Phase)			
6/7	5	5	6	6	4	3
			(End Second Phase)			
7/5	5	5	6	6	4	3

The vitamin A and carotene solutions were prepared in concentrations of one mg. per ml. according to the method of Bieri (3). The sheep were bled at weekly intervals and at the completion of this phase were again biopsied for liver samples.

The weekly blood samples obtained during the course of the experiment were analyzed for total serum protein, plasma vitamin A,

hematocrit, and electrophoretic separation of serum protein fractions. Total serum protein was determined with the Bausch & Lomb refractometer Model ABBE-3L according to the procedure outlined by Sunderman (47) and plasma vitamin A by the method of Kimble (25). Percent blood cell volume was obtained by the micro hematocrit technique and was read on an International rotary micro capillary reader. Serum proteins were fractionated using the Spinco Model R paper electrophoresis cell, Spinco Duostat and Model RB Analytrol. The liver samples taken at the end of each phase were analyzed for vitamin A and carotene concentration by the method of Gallup and Hoefer (17).

In addition to the above information the sheep were weighed initially and at the end of each phase of the experiment. The rations were also analyzed for crude protein and carotene content.

The experiment was set up as a single replication of a completely randomized design and the data were subjected to an analysis of variance as outlined by Snedecor (46).

RESULTS AND DISCUSSION

Phase I (4/19 - 6/7). When the experiment was initiated the only symptoms of vitamin A deficiency observed in the sheep were general excitability and occasional cases of muscular incoordination. These observations are in agreement with Klosterman et al. (26) who found no clinical symptoms in vitamin A depleted lambs, except those mentioned above.

The group fed the protein adequate ration had initial plasma vitamin A levels averaging 19.3 mcg. % and the protein depleted group averaged 18.4 mcg. % plasma vitamin A (Table 2).

Table 2. Influence of Dietary Crude Protein Level, Carotene and Vitamin A Administration on Blood Plasma Vitamin A.

Phase	<u>15.2% Crude Protein Ration</u>				<u>5.5% Crude Protein Ration</u>			
	Control	Oral Carotene	Oral Vitamin A	Avg.	Control	Oral Carotene	Oral Vitamin A	Avg.
Means (mcg. %) with Standard Errors ^a								
Initial	22.1 ± 3.6	15.6 ± 3.6	20.1 ± 3.6	19.3	19.1 ± 3.6	17.2 ± 3.6	19.0 ± 3.6	18.4
Phase I	24.9 ± 4.4	14.3 ± 4.4	17.1 ± 4.0	18.7	14.7 ± 4.0	14.5 ± 4.9	11.5 ± 5.6	13.9
Phase II	24.3 ± 4.2	26.6 ± 4.2	36.8 ± 3.8	29.7	13.9 ± 3.8	24.1 ± 4.7	27.9 ± 5.4	20.3

^a For number of animals per treatment see Table 1.

After the 50-day protein depletion period the sheep fed the protein adequate ration had an average plasma vitamin A level of 18.7 mcg. % while the average plasma vitamin A level in the protein depleted

group had fallen to 13.9 mcg. %. This average difference of approximately 5 mcg. % between the two groups was not statistically significant.

During the course of the experiment the values observed in the total serum proteins and serum albumin indicated a consistent decline in all treatment groups while the serum globulin values showed an overall increase. In addition to this general relationship in the change in the blood protein constituents significant treatment effects were observed. A possible explanation for this general shift in blood proteins may be associated with the stress due to the steady increase in ambient temperature during the experiment. The semi-monthly mean high temperatures from April 15, 1960 to July 15, 1960 are as follows:

April 15 - 30	82.1°
May 1 - 15	92.7°
" 16 - 31	93.6°
June 1 - 15	100.4°
" 16 - 30	108.5°
July 1 - 15	103.3°

Total serum protein values for the protein adequate and depleted groups were 6.25 g. % and 5.89 g. %, respectively (Table 3). This dif-

Table 3. Influence of Dietary Crude Protein Level, Carotene and Vitamin A Administration on Total Blood Serum Protein.

<u>15.2% Crude Protein Ration</u>				<u>5.5% Crude Protein Ration</u>			
Phase	Control	Oral Carotene	Oral Vitamin A	Avg.	Control	Oral Carotene	Oral Vitamin A
Means in g. % with Standard Errors ^a							
Initial	6.62 ±0.17	6.71 ±0.17	6.52 ±0.17	6.61	6.33 ±0.17	6.51 ±0.17	6.23 ±0.17
Phase I	6.21 ±0.24	6.63 ±0.24	5.97 ±0.22	6.25	5.86 ±0.22	5.85 ±0.27	5.98 ±0.31
Phase II	6.25 ±0.20	6.57 ±0.20	6.57 ±0.18	6.47	6.00 ±0.18	6.19 ±0.22	6.82 ±0.26
							6.35
							5.89
							6.25

^a For number of animals per treatment see Table 1.

ference in the depression of total serum protein in the protein depleted sheep was not found to be statistically significant.

Hematocrits taken one week before the end of the first phase showed significantly lower values ($P < .05$) for the protein depleted sheep, in agreement with the investigations of Peo et al. (41) with protein depleted baby pigs.

The protein depleted group demonstrated a highly significant ($P < .01$) drop in serum albumin as compared with the sheep on the protein adequate ration (Table 4). This pattern of alteration in the

Table 4. Influence of Dietary Crude Protein Level, Carotene and Vitamin A Administration on Blood Serum Albumin.

Vitamin A Administration on Blood Serum Albumin.								
15.2% Crude Protein Ration				5.5% Crude Protein Ration				
Phase	Control	Oral Carotene	Oral Vitamin A	Avg.	Control	Oral Carotene	Oral Vitamin A	Avg.
Means (g. %) with Standard Errors ^a								
Initial	3.91 ±0.13	3.84 ±0.13	3.78 ±0.13	3.84	3.74 ±0.13	3.74 ±0.13	3.63 ±0.13	3.70
Phase I	3.31 ±0.16	3.56 ±0.16	3.16 ±0.15	3.33	2.89 ±0.15	2.88 ±0.18	2.84 ±0.21	2.88
Phase II	2.83 ±0.14	3.09 ±0.14	3.13 ±0.13	3.03	2.82 ±0.13	2.72 ±0.16	2.63 ±0.19	2.75

^a For number of animals per treatment see Table 1.

serum protein fractions supports the earlier research of Klosterman et al (27) with pregnant ewes and Peo et al. (41) working with protein depleted baby pigs. However, this latter research did not reveal a drop in total serum proteins associated with protein depletion.

There was a definite trend toward elevated globulin levels in the protein depleted sheep although this alteration in serum globulins was not statistically significant. The treatment effects upon serum globulins are shown in Table 5.

Table 5. Influence of Dietary Crude Protein Level, Carotene and Vitamin A Administration on Blood Serum Globulins.

<u>15.2% Crude Protein Ration</u>				<u>5.5% Crude Protein Ration</u>			
Phase	Control	Oral	Oral	Avg.	Control	Oral	Oral
		Carotene	Vitamin A			Carotene	Vitamin A
Means (g. %) with Standard Errors ^a							
Initial	2.71	2.87	2.74	2.77	2.59	2.77	2.60
	±0.16	±0.16	±0.16		±0.16	±0.16	±0.16
Phase I	2.90	3.07	2.81	2.92	2.97	2.97	3.14
	±0.26	±0.26	±0.24		±0.24	±0.30	±0.34
Phase II	3.42	3.48	3.44	3.44	3.18	3.47	4.19
	±0.22	±0.22	±0.20		±0.20	±0.24	±0.28

^a For number of animals per treatment see Table 1.

At the end of the first phase 5 sheep on the low protein ration and 2 sheep on the adequate protein ration died at the time of liver biopsy. The higher death loss in the protein depleted group may have been attributable, in part, to the combined effects of protein depletion and vitamin A deficiency. Analysis of the liver samples taken at the end of this phase revealed no relationship between level of protein in the diet and hepatic vitamin A storage (Table 6). Confirmation of this finding was not complete since the initial liver stores were not known.

Table 6. Influence of Dietary Crude Protein Level, Carotene and Vitamin A Administration on Hepatic Vitamin A Storage.

<u>15.2% Crude Protein Ration</u>				<u>5.5% Crude Protein Ration</u>			
Phase	Control	Oral	Oral	Avg.	Control	Oral	Oral
		Carotene	Vitamin A			Carotene	Vitamin A
Means (mcg. per g.) with Standard Errors ^a							
Phase I	15.2	5.1	16.0	11.9	9.4	6.3	7.0
	±6.6	±6.0	±6.0		±6.0	±6.6	±6.0
Phase II	11.0	8.3	22.4	14.9	9.6	9.0	7.7
	±8.4	±9.3	±7.6		±7.6	±9.3	±10.8

^a For number of animals per treatment see Table 1.

At the start of the experiment body weights of the protein adequate and deficient sheep averaged 133 lbs. and 134 lbs., respectively. At the end of the 50-day depletion period the protein deficient sheep averaged 129 lbs., a loss of 5 lbs. per head, while the average weight of the protein adequate animals remained at 133 lbs.

Phase II (6/7 - 7/5). During this period sheep on both the protein deficient and protein adequate rations received daily oral doses of vitamin A or carotene. Within each of the 2 protein levels 1/3 of the sheep served as controls, 1/3 received 25 mcg. vitamin A acetate per kg. body weight daily, and 1/3 received 25 mcg. beta carotene per kg. body weight daily.

The serum albumin of the protein depleted animals continued at a significantly lower level ($P < .05$) than that of the sheep on the protein adequate ration (Table 4). The animals fed the adequate protein ration and given vitamin A or carotene showed less depression in serum albumin than any other groups while the sheep on the low protein ration given oral doses of vitamin A or carotene demonstrated the greatest lowering in serum albumin. The serum globulin levels increased from 21-61% over the initial levels in all treatment groups and while the animals on the low protein diet continued to show a trend toward raised serum globulin levels, the increase was not statistically significant (Table 5).

The plasma vitamin A levels of all sheep receiving oral administrations of either vitamin A or carotene were significantly higher than the controls ($P < .05$) regardless of the level of protein in the ration. Table 2 indicates that the rise in plasma vitamin A over the

initial levels during this phase was 82% in the sheep receiving vitamin A, on the protein adequate ration and 47% in similarly treated sheep on the protein deficient ration. This same relationship was noted in the sheep orally supplemented with carotene with the adequate protein group increasing 70% over initial levels and the sheep on the low protein ration showing a corresponding 40% increase. The plasma vitamin A levels in the protein adequate controls were 10% higher than initial levels and 27% lower than initial levels in the protein depleted controls.

The total serum protein levels were significantly higher in the sheep receiving vitamin A or carotene ($P < .05$) compared with the control animals, regardless of the level of protein in the ration (Table 3). Serum protein levels in both the protein adequate and protein deficient sheep receiving vitamin A rose slightly over initial levels while the total serum protein in all other treatment groups declined from 2-6%.

At the end of the second phase 2 animals were lost at the time of liver biopsy, one from each protein level. Hepatic vitamin A storage rose highest in both of the carotene supplemented groups, measured in percent change over the stores on 6/7. Both of the groups receiving vitamin A were next in amount of hepatic vitamin A storage while the the normal control animals showed a decline in storage and the protein depleted controls exhibited essentially no change in liver vitamin A stores (Table 6).

The effect of protein deficiency as measured at the end of the

first phase was evidenced throughout the experimental data. Weight loss, lowered hematocrit, lowered total serum protein, as well as an alteration in serum protein fractions were changes observed in the protein deficient animals which supported the findings in earlier research. An interrelationship between the level of dietary protein and vitamin A metabolism was suggested in that the protein deficient sheep showed lower plasma vitamin A levels and hepatic vitamin A reserves than the sheep fed the protein adequate ration. The lower blood and liver vitamin A values in the protein deficient sheep point toward a possible antagonism between low dietary protein intake and the efficiency of utilization of vitamin A reserves. This type of interaction is particularly undesirable in animals with low hepatic vitamin A reserves since it is essential that these reserves be used as efficiently as possible to delay the onset of vitamin A deficiency symptoms. Work by Mayer and Krehl (30) and McLaren (31) with young rats, deficient in vitamin A, found, conversely, that protein deficiency had a sparing effect on hepatic vitamin A reserves and high protein diets hastened the depletion of liver stores. A possible explanation for the discrepancy between the findings in the rat studies and in those reported in this thesis might be a species and/or age difference in protein-vitamin A metabolism between young, growing rats and mature ewes.

The higher plasma and liver vitamin A values indicate that the sheep on the adequate protein ration utilized the vitamin A and carotene doses more efficiently than the sheep fed the low protein ration. This observation is in agreement with the work of Baumann (2) and

Leutskii (29) who found that protein deficient rats stored less liver vitamin A than those fed an adequate level of dietary protein.

Due to the comparatively low level of vitamin A and carotene administered during the second period hepatic vitamin A reserves did not increase proportionally as much as the plasma levels. This finding is consistent with the theory that the optimum plasma vitamin A level is first satisfied with any excess vitamin A being stored in the liver (32). It also supports the National Research Council recommendation (36) that from 3 to 5 times the minimum daily requirement of vitamin A is necessary to achieve any appreciable liver storage.

The higher total serum protein levels found in the sheep supplemented with vitamin A or carotene fed either level of protein does not support the observations of Brown and Morgan (5) or Erwin et al. (13). The former researchers found that young rats, deficient in vitamin A, did not use protein as efficiently as those supplemented with vitamin A but were not able to produce the same effect in mature rats which were vitamin A deficient. They theorized that vitamin A is more essential for growth of tissue protein than for maintenance. Erwin et al. (13) were unable to demonstrate a rise in total serum protein in steers associated with carotene supplementation although they did observe a rise in serum albumin and a reduction in serum globulins.

No outward symptoms of vitamin A deficiency were observed in the animals during the course of the research, with the exception of the general nervousness and occasional cases of muscular incoordination under stress which were evident at the initiation of the experiment.

SUMMARY

The influence of protein depletion on vitamin A and carotene utilization by vitamin A deficient sheep was studied in 36 mature ewes and wethers of mixed breeding.

It was found that a highly significant relationship exists between the level of dietary protein and the level of serum albumin with the protein deficient sheep demonstrating lower values than those sheep on the protein adequate ration. Protein deficiency also resulted in lowered total serum protein, lowered hematocrit and weight loss in the depleted animals. Plasma and liver vitamin A values were found to be lower in the protein depleted animals indicating less efficient utilization of vitamin A reserves in protein deficiency.

Vitamin A and carotene administration was more effective in the sheep fed the adequate protein ration as evidenced by higher plasma vitamin A levels and hepatic vitamin A storage.

Finally, total serum protein levels were higher in the vitamin A and carotene supplemented sheep when compared with the control animals, regardless of the level of protein in the ration.

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