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Methods of Supplementing Carotene and Vitamin A to Vitamin A-Depleted Sheep

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The vitamin A requirement of sheep has not received the attention in recent years as has that of beef cattle. The need for definitive requirements is lessened because of the unique ability of the sheep to require extended periods of time, often a year or more, to develop vitamin A deficiency symptoms when fed diets low in vitamin A activity. Also, the conversion of beta-carotene to vitamin A by sheep has been found to be about 1.75 times greater than for cattle. Many experiments have shown that rate of growth is not impaired despite serious depletion of vitamin A stores caused by feeding low-carotene or vitamin A diets.

Methods of administrating vitamin A other than through a daily supplement may at times provide a more practical and economical means of meeting the requirements for the vitamin. Fattening beef cattle not depleted of vitamin A and finished on a low-carotene diet had varied responses to methods of supplementation. Supplementing the diet with 10,000 International Units (IU) of vitamin A or an equivalent total at 2-week intervals gave similar results as measured by plasma and liver vitamin A levels. Voluntary intake of a free-choice mineral containing vitamin A was low and resulted in lower plasma and liver levels of the vitamin. A massive single injection of vitamin A at the start of the feeding trial resulted in liver stores that were higher than those of the other methods investigated.

The efficiency by which carotenes are utilized and the extent of conversion to vitamin A in the sheep is fairly well documented in research reports. Oral or intramuscular injections of carotene appear to be rapidly metabolized and in most cases lead to elevated blood plasma levels of vitamin A. Similar responses have not been consistently demonstrated for liver storage of the vitamin.

The primary objective of these experiments was to study the effectiveness of different methods of supplementing vitamin A to vitamin A-depleted lambs as measured by growth, blood plasma levels and liver stores of vitamin A. The second objective was to determine effect of oral and injection methods of carotene administration on subsequent vitamin levels in blood plasma, liver, heart and kidney tissue. Responses were monitored over a period of about 4 weeks.

Prepared for Sheep Field Day, June 10, 1977.

Experimental Procedure

Experiment 1

Forty-seven wether lambs averaging 65 lb were allotted to four pens with 15 lambs per pen. During a vitamin A depletion phase, the lambs were fed a low-carotene diet consisting of a full feed of whole, rolled oats. A mineral mixture composed of equal parts of trace mineral salt and dicalcium phosphate was offered free choice. Samples of jugular blood were collected initially on May 20, 1975, at 49 days (July 8, 1975) and at 197 days (December 3, 1975). The blood serum was analyzed for carotene and vitamin A. Seven lambs were randomly selected for slaughter at the end of the depletion phase (December 3, 1975). Samples of liver tissue were collected for carotene and vitamin A analyses. Feed consumption and rate of growth during the vitamin A depletion were recorded.

For the repletion phase, 40 vitamin A-depleted sheep were randomly allotted to each of four pens with 10 sheep per pen. The sheep averaged 120 pounds in weight.

The four methods of supplementing vitamin A were as follows:

1. Conventional--supplement fed daily with vitamin A and mineral.
2. Intermittent--supplement with vitamin A top-dressed to the diet every 2 weeks, mineral free choice.
3. Vitamin A in mineral offered free choice.
4. Vitamin A injected at the start of the trial, mineral free choice.

The conventional supplement was fed at the rate of .3 lb per head daily. The supplement supplied 3,000 IU of vitamin A per animal. The top-dressed supplement was fed at a .3-lb rate, but the level of vitamin A was equal to 14 days of the daily feeding level at one time at the beginning of each 2-week period. Free-choice mineral was placed in boxes with vitamin A included in the mixture at a level of 102,000 IU per pound. Vitamin A was administered by intramuscular injection at the beginning of the feeding period in the amount of 300,000 IU. A supplement containing 89% ground corn and 11% ground limestone without vitamin A was fed at a rate of .3 lb per head daily with the intermittent free-choice mineral and injection methods of vitamin A supplementation.

Composition of supplements and mineral mixtures is shown in table 1.

The sheep were slaughtered at the end of a 91-day repletion phase and samples of blood and liver tissue were taken for carotene and vitamin A analyses.

Samples of feeds used in the trial were analyzed for carotene content.

Table 1. Vitamin and Mineral Supplements

Ingredient	Conventional	Intermittent top-dress	Free-choice mineral: With vitamin A[a]	Free-choice mineral: Without vitamin A
	%	%	%	%
Ground corn	88.927	87.973	--	--
Trace mineral salt	--	--	99.250	100.00
Ground limestone	11.000	11.000	--	--
Vitamin A premix[b]	.073	1.027	.750	--

[a] Contains 225 IU vitamin A per gram.
[b] Contains 30,000 IU per gram.

Experiment 2

Fifty-six lambs were maintained on a low-carotene diet without supplemental vitamin A for 259 days. The lambs averaged 46 lb initially and were fed rolled, whole oats and offered a mineral mixture consisting of equal parts trace mineral salt and dicalcium phosphate free-choice. Samples of jugular blood and liver tissue were obtained at time of slaughter. Lambs were sacrificed at the start of the depletion period (September 23, 1975), at 74 days (December 8, 1975) and at 259 days (June 9, 1976) and the samples frozen for carotene and vitamin A analyses. The lambs were weighed periodically during the depletion period and feed consumption was recorded.

The remaining 33 sheep averaged 100 lb at the end of the depletion period. The sheep were allotted to three pens of 11 head each. Three treatments were as follows:

1. Control--rolled whole oats and a supplement to provide 2,000 IU of vitamin A per head daily.

2. Injected--control diet plus an intramuscular (IM) injection of an aqueous beta-carotene solution.

3. Drenched--control diet plus an intraruminal (IR) drench of aqueous beta-carotene solution.

The supplement was fed to all treatment groups at .2 lb per head per day. Composition of the supplement was 61% ground oats, 22% ground limestone, 8.8% trace mineral salt and 6.6% potassium chloride with aureomycin and vitamin A[1] added to provide 75 mg and 2,000 IU per head per day, respectively.

[1] The vitamin A supplied by Diamond Shamrock Chemical Company, Cleveland, Ohio, courtesy of Gary Vannorsdel.

One group received a 2 ml intramuscular (IM) injection of an aqueous solution of beta-carotene[2]. The solution consisted of an emulsion containing 3.6% beta-carotene diluted 1:2 with water and provided 18 mg beta-carotene per milliliter. Each lamb received 36 mg of beta-carotene or enough to meet about one-half the National Research Council's (NRC) recommendation for a 110-lb sheep for a 30-day period.

A second group of sheep was administered an intraruminal (IR) dose of beta-carotene. The drench consisted of a water-dispersible material[2] containing 10% carotene activity. The final solution contained 3.6 mg beta-carotene per milliliter. Each sheep received 20 ml of drench deposited into the rumen by stomach tube. This amounted to about 72 mg of total carotene or enough administered at one time to be equal to the NRC recommended allowance for a 30-day period.

The sheep were slaughtered periodically after carotene administration. Three sheep were slaughtered from each treatment group 6 days after the treatment was administered to obtain heart, liver and kidney tissues for vitamin A analysis. Blood samples from the slaughtered animals and the remaining animals were collected and frozen for carotene and vitamin A analyses. Four animals from each treatment were slaughtered at 13 days and four at 27 days after the administration of carotene. Blood and tissue samples were collected at slaughter.

Results

Experiment 1

Performance and blood measurements during the 197-day vitamin A depletion period are presented in table 2. Weight gains and levels of blood and liver vitamin A for all lambs are shown in figure 1. Body weight gains were satisfactory for a diet of this type. Daily gains averaged .269 to .290 lb per head daily. Blood levels of vitamin A dropped from an average of 27 mcg/100 ml at the start of the experiment to 9 mcg/100 ml at the end of 197 days. Liver vitamin A stores were not determined initially. However, seven lambs were slaughtered at the end of the depletion phase to provide base values with respect to vitamin A storage and to evaluate the effectiveness of methods of vitamin A supplementation. By the end of the depletion period the concentration of the vitamin in the liver was down to 1.56 mcg per gram. This level would be considered well below that representing normal vitamin A nutrition. Intake of dietary carotenoids averaged .82 mg per lamb daily.

Results of the supplementation or repletion phase of the experiment are presented in table 3. Differences in daily gain, daily feed and feed efficiency show some variations between methods of vitamin A supplementation. However, deifferences in magnitude shown probably should be expected with the numbers of lambs used. Therefore, method of vitamin A supplementation apparently had little, if any, effect on feedlot performance over the 91-day supplementation period.

[2] The beta-carotene used in this study was supplied by Hoffman-LaRoche, Inc., Nutley, New Jersey, courtesy of Drs. C. R. Adams and A. A. Kurnick.

The blood plasma and liver concentrations of carotene and vitamin A are presented in table 4. Carotene values for blood and liver tissue were low initially and throughout the repletion period. Blood and liver carotene concentrations are normally quite low in sheep. Vitamin A concentrations in blood plasma averaged 12.2 mcg/100 ml in lambs slaughtered and 8 to 9 mcg/100 ml in lambs retained for supplementation and sampled by vena puncture. Final blood values showed an increase to 34 mcg/100 ml in sheep supplemented with vitamin A daily, 24 mcg/100 ml in sheep fed the top-dressed supplement and 21 mcg/100 ml in those injected with the vitamin. Sheep receiving the free-choice mineral had the lowest level of blood plasma vitamin A, 18 mcg/100 milliliters.

Vitamin A concentrations in liver tissue increased from 1.56 mcg/g to about 7 mcg/g during the 91-day depletion period with all methods of supplementation except the free-choice mineral method which remained at the depletion level. The calculated average daily intake of preformed vitamin A (table 4) was 2983 IU per head for the daily supplement and 2997 IU per head for the supplement fed at 2-week intervals. Intake of free-choice mineral resulted in a daily intake of 2247 IU per head. The intake of the free-choice mineral supplement was lower than expected giving the lower intake in comparison to the other methods of supplementation. The variable pattern of free-choice mineral consumption is a serious objection to this method of vitamin A supplementation. In addition, the vitamin is less stable in mineral mixes. Some greater loss of activity in the mineral supplement in comparison to the other supplements may be indicated in view of the lack of any apparent response even though the daily intake of mineral was less than expected.

Injected vitamin A, when averaged over the 91-day period, resulted in an allowance of 3297 IU per head daily. While slightly higher than the average daily intake through daily or 2-week supplementation, liver values were at similar levels from these three methods of supplementation.

Intake of carotenoids from the diet is reported in table 4. Values were in the order of 1 mg per head daily and were determined by the Carr-Price Chemical method. This method does not indicate the potential vitamin A activity of the carotenes.

Experiment 2

The results of the 259-day vitamin A depletion period are shown in figure 2. Rates of growth were satisfactory with the rolled, whole oat diet that was fed. Gains ranged from .217 to .233 lb per head per day. Blood plasma levels of vitamin A generally declined over the period but were not lowered to the extent as was observed in experiment 1. Liver vitamin A concentrations of 127 mcg/g initially would be considered to be high. However, levels had declined to 15 mcg/g by 74 days and down to 1.4 mcg/g at 259 days. Intake of carotenoids from the diet averaged .58 mg per head daily.

The effects of intramuscular or intraruminal injections of beta-carotene on carotene and vitamin A concentrations in blood, liver, heart and kidney tissues are presented in tables 5 and 6. Because different quantities of beta-carotene were administered (36 mg total for intramuscular and 72 mg total for intraruminal injections), comparisons between the two methods of injection are not possible. However, the injection treatments are evaluated in terms of the control treatment.

Carotene analyses (table 5) from all samples resulted in low values. No apparent differences between treatment groups were observed from either blood, liver, heart or kidney tissues. The carotene treatments did not appear to have any effect on carotene values with time after administration. Liver, heart and kidney tissues have much lower levels of carotene than blood. Carotene concentrations of this magnitude are difficult to determine with accuracy due to the nature of the analytical procedures used.

Concentrations of vitamin A in blood, as presented in table 4, show that sheep given an aqueous beta-carotene solution intramuscularly had only slightly higher levels of the vitamin than control sheep through 13 days post-administration. In contrast to control animals, blood vitamin A levels increased rapidly with the intraruminal dose of beta-carotene solution. Levels reached 40 mcg/100 ml by 6 days and then declined to 30 mcg/100 ml at 13 days and dropped to 22 mcg/100 ml at 27 days.

Liver tissue, the principle site of body reserves of vitamin A, contained a relatively low level of the vitamin, 1.355 mcg/g, at the time of administration of beta-carotene. Intramuscular injections increased the vitamin A concentrations to 1.94 mcg/g at 6 days, but then levels dropped off and were comparable to the controls by the end of the fourth week. With the intraruminal injections, vitamin A increased to a peak concentration of 6 mcg/g at 13 days and then declined to 3 mcg/g at 27 days. Liver vitamin A levels were four to five times greater than those of the controls.

Heart and kidney tissues were found to contain very low levels of vitamin A. No important differences were observed that were related to carotene treatments.

The sheep in each of the three treatment groups received 2000 IU of vitamin A per head daily in a supplement. This level of supplementation was believed to meet the daily vitamin A requirements but would not allow for any appreciable amount of storage. The addition of vitamin A activity in the form of beta-carotene from the injections would be expected to be converted to vitamin A and therefore would appear in the blood and liver tissue. This was indeed the case with the intraruminal injections, where a total of 72 mg of beta-carotene were given. The lower amount of carotene (36 mg) used in the intramuscular injections did not appear to be converted to vitamin A in the same manner. Both the level administered and the rate of conversion may have influenced the response obtained with this treatment.

Summary

Feedlot lambs fed a low-carotene diet for periods up to 259 days gained rather well, but blood levels and liver stores of vitamin A were greatly diminished. Deficiency symptoms, however, were not observed in either of two experiments that involved a total of 103 lambs.

Methods of administering supplemental vitamin A and carotene to vitamin A-depleted sheep were compared. Supplementing the diet with a level of about 3000 IU of vitamin A daily provided adequate vitamin A nutrition as measured by blood and liver levels of the vitamin. Vitamin A administered in an amount equivalent to the total daily level but at 2-week intervals or given as a single dose (3297 IU daily) likewise maintained adequate blood plasma and liver levels.

Allowing sheep a free-choice mineral containing vitamin A resulted in poor mineral consumption and a lowering of vitamin A intake and consequently lower blood concentrations of vitamin A. Liver storage levels remained at depletion levels, indicating that the lower intake and possible reduced activity did not provide an adequate amount to allow liver storage.

Injections of a total of 36 mg beta-carotene intramuscularly increased vitamin A concentrations in blood and liver tissues only slightly, even though enough preformed vitamin A was fed to meet the daily requirements. Intraruminal injections of 72 mg beta-carotene resulted in rapid increases up to 13 days in blood levels of vitamin A. Liver levels of vitamin A were four to five times those of the control animals and remained elevated for up to 2 weeks after dosing before declining.

Table 2. Performance and Blood Measurements of Sheep Fed a Vitamin A Deficient Diet (May 19 to December 2, 1975--197 Days)

	Rolled oats and free-choice minerals			
Lot	45	46	47	48
No. of animals[a]	13	10	12	12
Weight, lb				
Initial (May 19)	64.9	63.8	64.3	65.8
June 23	70.9	69.2	71.3	70.8
October 7	111.3	115.3	108.6	110.9
December 2	120.3	121.0	117.4	122.2
Avg daily gain, lb	.281	.290	.269	.286
Avg daily ration, lb				
Rolled oats	2.36	2.79	2.57	2.53
Mineral mixture[b]	.046	.076	.057	.049
Total	2.406	2.866	2.627	2.579
Feed/100 lb gain, lb				
Rolled oats	840	962	952	886
Mineral mixture	17	26	21	17
Total	857	988	973	903
Blood carotene, mcg/100 ml				
May 20		Not determined		
July 8		Not determined		
December 3	4.24	4.89	4.70	4.20
Blood vitamin A, mcg/100 ml				
May 20	28.56	28.54	26.21	26.93
July 8	22.57	22.78	28.03	27.95
December 3	9.02	9.42	8.09	9.29

[a] Animals were removed from the lots due to causes not related to depletion of vitamin A.

[b] Equal parts of trace mineral salt and dicalcium phosphate.

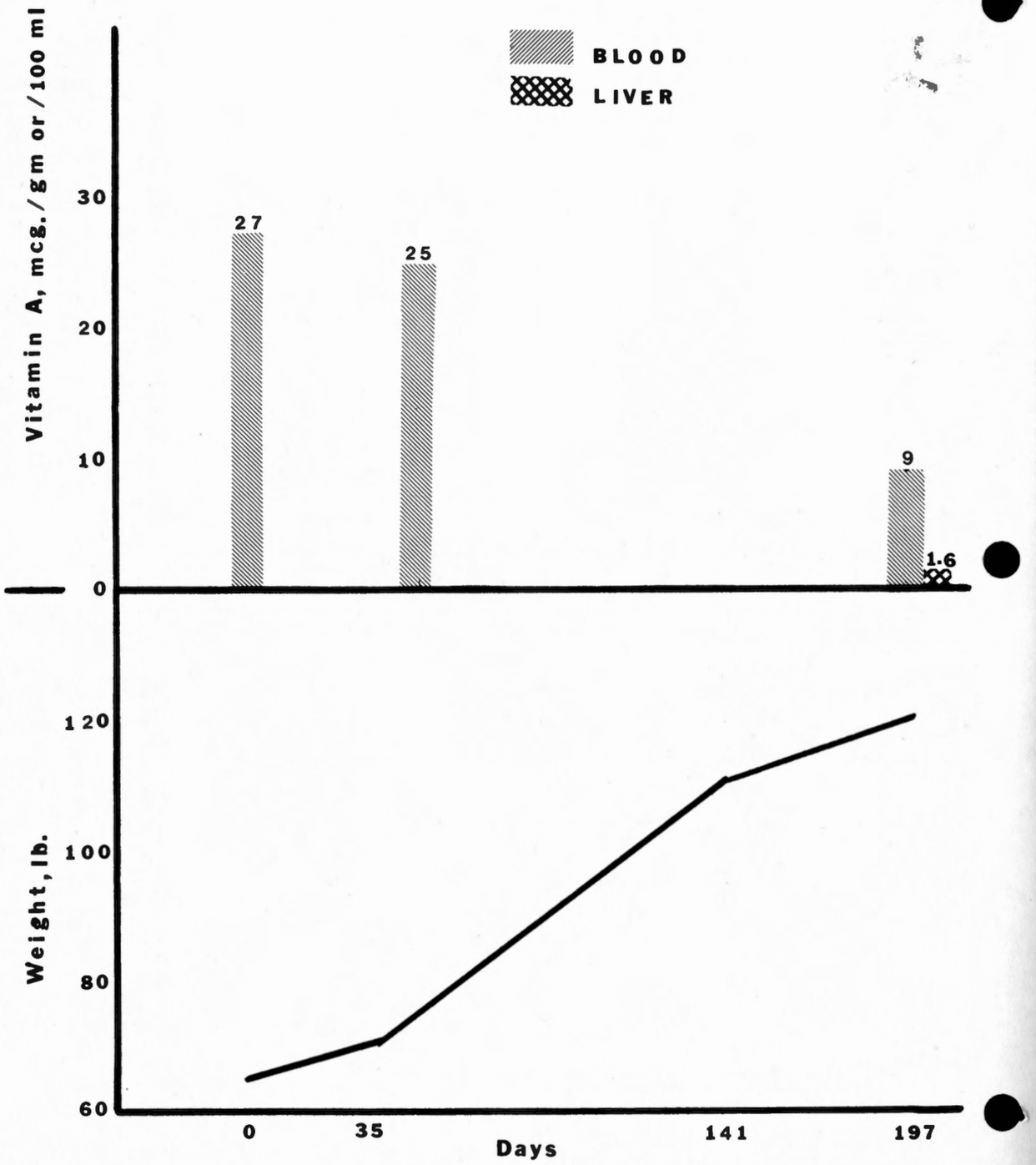

FIGURE 1. VITAMIN A DEPLETION—RATE & WEIGHT GAIN EXPERIMENT 1

Table 3. Feedlot Performance of Vitamin A-Depleted Sheep and Methods of Supplementation (December 2, 1975, to March 2, 1976--91 Days)

	Treatments with vitamin A			
	Daily supplement	Top-dressed at 2-week intervals	Minerals free-choice	Injection
No. of animals	10	10	10	10
Initial wt., lb	116.0	116.0	116.2	116.2
Final wt., lb	142.8	143.9	145.3	146.5
Avg daily gain, lb	.295	.307	.320	.333
Avg daily feed, lb				
Rolled oats	2.96	2.97	2.87	2.96
Supplement with vitamin A	.287	.013	--	--
Supplement without vitamin A	--	.297	.287	.293
Trace mineral salt	.033	.022	.022	.022
Feed/100 lb gain, lb				
Rolled oats	1005	969	898	889
Supplement with vitamin A	98	7	--	--
Supplement without vitamin A	--	88	90	88
Trace mineral salt	11	4	7	7

Table 4. Blood Plasma and Liver Concentration of Carotene and Vitamin A

	Treatments with vitamin A			
	Daily supplement	Top-dressed at 2-week intervals	Minerals free-choice	Injection
Avg vitamin A intake, IU/day	2983	2997	2247	3297
Avg carotenoids consumed, mg/day	1.11	1.08	1.04	1.07
Blood carotene, mcg/100 ml				
Slaughtered animals[a]			4.60	
Initial (12-3-75)	4.20	4.70	4.89	4.24
Final (3-2-76)	4.09	5.42	5.18	5.04
Blood vitamin A, mcg/100 ml				
Slaughtered animals			12.20	
Initial (12-3-75)	9.29	9.42	8.09	9.02
Final (3-2-76)	34.38	24.03	18.26	21.08
Liver carotene, mcg/g				
Slaughtered animals			.42	
Final	.39	.40	.41	.42
Liver vitamin a, mcg/g				
Slaughtered animals			1.56	
Final	7.10	6.39	1.54	6.77

[a] Seven sheep slaughtered at start of repletion experiment, December 5, 1975.

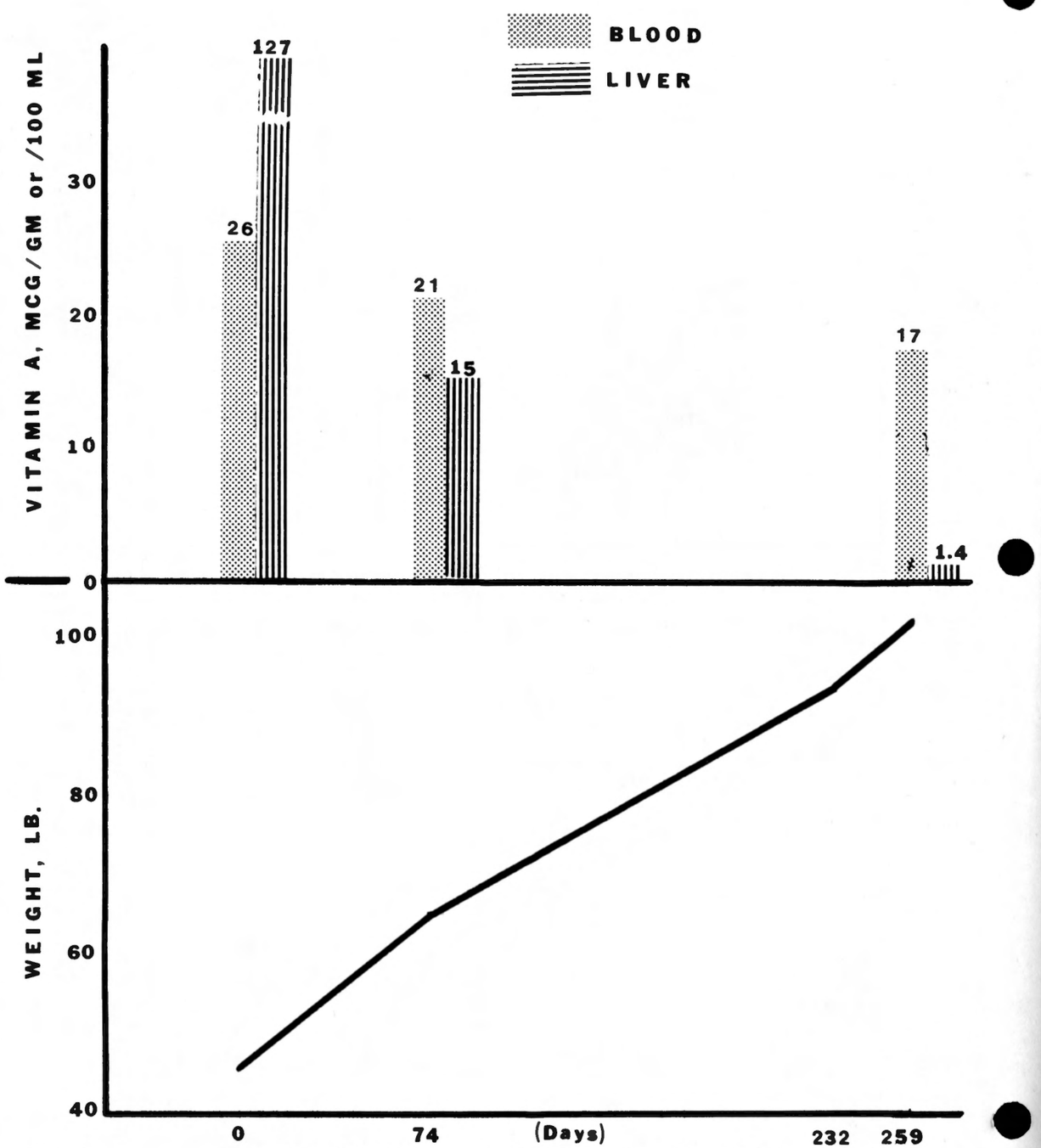

FIG. 2 VITAMIN A DEPLETION – RATE & WEIGHT GAIN EXPERIMENT 2

Table 5. Carotene Concentrations in Blood and Tissues of Sheep

Measurement[a]	Days after administration	Control	Carotene injected (IM)[b]	Carotene drenched (IR)[c]
Blood serum, mcg/100 ml	0	4.03 (11)	3.58 (11)	4.65 (11)
	6	3.49 (10)	3.92 (11)	3.49 (11)
	13	2.95 (8)	3.60 (8)	2.81 (8)
	27	3.50 (4)	3.41 (4)	2.93 (4)
Liver, mcg/g	0	.400 (4)	--	--
	6	.386 (3)	.307 (3)	.470 (3)
	13	.330 (4)	.297 (4)	.328 (4)
	27	.315 (4)	.297 (4)	.367 (4)
Heart, mcg/g	0	.297 (4)	--	--
	6	.278 (3)	.312 (3)	.205 (3)
	13	.317 (4)	.248 (4)	.263 (4)
	27	.306 (4)	.229 (4)	.291 (4)
Kidney, mcg/g	0	.129 (4)	--	--
	6	.139 (3)	.133 (3)	.130 (3)
	13	.156 (4)	.148 (4)	.159 (4)
	27	.108 (4)	.109 (4)	.103 (4)

[a] Values in parentheses indicate number of observations used to calculate average.
[b] IM = intramuscular.
[c] IR = intraruminal.

Table 6. Vitamin A Concentrations in Blood and Tissues of Sheep

Measurement[a]	Days after administration	Control	Carotene injected (IM)[b]	Carotene drenched (IR)[c]
Blood serum, mcg/100 ml	0	10.29 (11)	12.49 (11)	13.74 (11)
	6	15.59 (10)	18.22 (11)	40.45 (11)
	13	15.75 (8)	17.06 (8)	29.82 (8)
	27	11.23 (4)	10.64 (4)	21.76 (4)
Liver, mcg/g	0	1.355 (4)	--	--
	6	.994 (3)	1.944 (3)	4.398 (3)
	13	1.078 (4)	.814 (4)	6.056 (4)
	27	.721 (4)	.879 (4)	2.997 (4)
Heart, mcg/g	0	.137 (4)	--	--
	6	.202 (3)	.143 (3)	.150 (3)
	13	.137 (4)	.142 (4)	.141 (4)
	27	.142 (4)	.181 (4)	.176 (4)
Kidney, mcg/g	0	.254 (4)	--	--
	6	.235 (3)	.215 (3)	.443 (3)
	13	.220 (4)	.244 (4)	.229 (4)
	27	.219 (4)	.234 (4)	.274 (4)

[a] Values in parentheses indicate number of observations used to calculate average.
[b] IM = intramuscular.
[c] IR = intraruminal.