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Several researchers have demonstrated that dietary nitrate reduced liver stores of vitamin A in ruminants. However, it has not been shown whether the effect is due to a more rapid depletion of existing vitamin A stores or to a lowered hepatic deposition of the vitamin.

Two experiments were conducted to determine the effects of nitrate on plasma vitamin A, hepatic vitamin A and performance of lambs during a growing period (experiment 1) and vitamin A depletion and repletion periods (experiment 2).

Procedures

Experiment 1

One hundred twenty black-faced ewe lambs were used in this growth study under conditions of vitamin A depletion and vitamin A or carotene supplementation with and without nitrate supplementation. Treatments were replicated with 10 lambs per pen.

The control low-carotene ration without vitamin A supplementation contained 75% rolled oats, 16.5% ground corn grain, 5% soybean meal, 2.5% sodium chloride and 1% limestone. This all-concentrate mix was full-fed with 0.25 lb. daily of a protein supplement composed of 95.1% of a corn-soybean meal mix (3 parts corn to 1 part soybean meal), 2.5% sodium chloride and 1% limestone. The supplement was fortified with diethylstilbestrol and chlortetracycline to furnish 2 and 25 mg., respectively, per lamb daily.

Vitamin A was added to the supplement to provide 4,000 I.U. per lamb daily for the vitamin A supplemented group. For the carotene supplemented group, dehydrated alfalfa meal replaced an equal weight of the corn-soybean meal mix in the supplement to provide 8 mg. of carotene per lamb daily. The supplements were mixed at 6-week intervals and dehydrated alfalfa meal analyzed prior to each mixing to determine the amount necessary to give the 8 mg. of carotene in 0.25 lb. of supplement. The protein content of the supplement was essentially unchanged by substitution of dehydrated alfalfa meal for the corn-soybean meal mix.

[1]Taken largely from paper published in Journal of Animal Science, Vol. 27, No. 6, pages 1727-1733. 1968.

For the nitrate supplemented lambs, 2.5% sodium nitrate was substituted for the 2.5% sodium chloride in the concentrate mixtures and the supplements. This resulted in six dietary treatments - control, 4,000 I.U. vitamin A daily and 8 mg. carotene daily, each with and without 2.5% sodium nitrate.

The lambs were brought to full-feed on the non-nitrate control ration over a 3-week period. Thereafter, they were full-fed the experimental rations once daily. Trace mineral salt and a mineral mixture of 2 parts dicalcium phosphate, 2 parts limestone and 1 part trace mineral salt were offered free access. The lambs were drenched with thiabendazole and vaccinated for prevention of enterotoxemia.

Blood samples were obtained by jugular puncture initially and at approximately 3-week intervals thereafter for lambs fed the vitamin A depletion rations and at about 6-week intervals for those supplemented with vitamin A or carotene. Blood samples were analyzed for total hemoglobin, methemoglobin, plasma carotene and plasma vitamin A. One lobe of each liver was collected at slaughter and analyzed for carotene and vitamin A.

Some losses occurred during the course of the experiment from causes unrelated to experimental treatment. Weight gain data were calculated only for lambs completing the experiment. Feed consumption data were adjusted by subtracting an average value for each loss.

Experiment 2

Depletion period. Concurrently with experiment 1, another 12 pen of ewe lambs from the same source were fed the low-carotene control ration as in experiment 1, one-half of which received the ration with 2.5% sodium nitrate. These lambs were used to obtain more extensive data on vitamin A depletion as affected by nitrate and to provide lambs for subsequent use in the vitamin A repletion phase of experiment 2. The lambs were bled initially and near the end of the 136-day depletion period, and the blood analyzed for plasma vitamin A.

Repletion period. Upon termination of the vitamin A depletion period, four pens of lambs from each of the control and nitrate treatments were used to determine the effects of nitrate on vitamin A repletion rates of lambs. Two pens from each of the control and nitrate treatments were supplemented with 10,000 I.U. of vitamin A per lamb daily and the other two pens from each source were supplemented with 20 mg. of carotene from dehydrated alfalfa meal.

Rations were similar to experiment 1 except for the levels of vitamin A and carotene supplementation and the supplement was fed at 0.5 lb. per lamb daily. The levels of diethylstibestrol and chlortetracycline were adjusted to provide 2 and 25 mg. respectively, per lamb daily.

Blood samples were obtained initially, after 3 weeks and upon termination of the 43-day vitamin A repletion period. Livers were analyzed upon slaughter.

Results

No visible signs of nitrate toxicity were observed during these experiments. Average methemoglobin values were low and never exceeded 0.6 gm per 100 ml. of blood. Therefore, these values are not reported in the tables. Presence of carotene in the liver or blood plasma was negligible in all instances. This has been shown to be characteristic for sheep and values for carotene are not reported in the tables.

Experiment 1

No particular problems were encountered from feeding the all-concentrate rations to lambs. Rates of gain and feed consumption were considered satisfactory for these ewe lambs (table 1). The control ration supplied about 1 mg. carotene daily per lamb. Supplementing it with 4,000 I.U. of vitamin A or 8 mg. of carotene from dehydrated alfalfa meal did not significantly affect weight gains, feed consumption or feed efficiency during 136-day experiment.

Feeding of 2.5% sodium nitrate reduced weight gains. The non-nitrate vitamin A treatment group gained at a lower rate than the corresponding controls. Taking this into consideration, the similarity in weight gains for the vitamin A treatments with and without nitrate is not believed to indicate a protective effect for vitamin A or a difference in the protective effect of vitamin A and carotene.

Plasma vitamin A values after 26 days were higher than initial values for all treatments. Only small changes were observed thereafter until the 110-day bleeding. At this time and at 132 days, values declined for lambs not supplemented with vitamin A or carotene. This effect was more pronounced in the presence of nitrate. In the absence of nitrate, there were only small differences in final plasma vitamin A values between vitamin A and carotene treatments. In the presence of nitrate, plasma vitamin A values were higher for lambs fed vitamin A than for those fed carotene.

Average liver vitamin A levels at the end of the 136-day experimental period were lowest in each instance for the nitrate-fed lambs. Vitamin A or carotene supplementation resulted in higher liver stores of vitamin A. However, 8 mg. of carotene per lamb daily did not support liver vitamin A stores at as high levels as did 4,000 I.U. of vitamin A. Terminal liver vitamin A stores of lambs fed carotene with and without nitrate, respectively, were only 50 and 64% as high as for corresponding lambs fed vitamin A.

Experiment 2

Depletion period. A reduction in weight gain resulted from feeding rations with 2.5% sodium nitrate (table 2). Plasma vitamin A values tended to become lower during the depletion with the effect being greater for those fed nitrate.

Repletion period. Weight gains were low and there were only small differences among treatments during the 43-day vitamin A repletion period (table 3). However, this phase of the experiment was of short duration and the lambs were rather heavy at this time.

Plasma vitamin A increased after 21 days of supplementation with 10,000 I.U. of vitamin A or 20 mg. of carotene per lamb daily. After 43 days, there was no difference in plasma vitamin A between lambs supplemented with vitamin A or carotene in the absence of nitrate. However, the feeding of 2.5% sodium nitrate resulted in lower plasma vitamin A at the 43-day sampling period. This affect appeared to be more pronounced with the feeding of carotene than with vitamin A.

A comparison of repleted liver vitamin A values (table 3) and prior depleted values (table 1) indicates that some degree of hepatic vitamin A storage occurred during the repletion period. In the absence of nitrate, the increases in hepatic storage amounted to 16 and 9 mg. per gm. with the feeding of vitamin A and carotene, respectively. Increases in hepatic vitamin A stores in nitrate-fed lambs during this time were only 44% as high as the increases observed in lambs on corresponding non-nitrate treatments with a similar effect for lambs fed vitamin A and those fed carotene.

Based on increases in hepatic vitamin A stores during the 43-day repletion period, the 20 mg. of carotene with and without nitrate was only 57 and 56%, respectively, as effective as 10,000 I.U. of vitamin A in contributing to hepatic vitamin A stores. This relative effectiveness of carotene vs. vitamin A is higher than was associated with lower levels of supplementation in experiment 1.

Summary

Nitrate significantly reduced weight gains in lambs being depleted or receiving modest level of vitamin A or carotene supplementation, but it did not have the same effect during a repletion period when the lambs were considerably larger and making lower rates of gain.

Carotene fed at 20 mg. per head daily from dehydrated alfalfa meal was only 56 to 57% as effective as 10,000 I.U. of vitamin A in the repletion of hepatic vitamin A stores of depleted lambs. This relative effectiveness of carotene vs. vitamin A was higher than was associated with lower levels of supplementation in nondepleted lambs. The feeding of 2.5% sodium nitrate did not appear to influence the relative effectiveness of carotene compared to vitamin A.

Lower plasma vitamin A and lower liver vitamin A values accompanied the feeding of nitrate to lambs supplemented with either carotene or vitamin A following vitamin A depletion. These effects of nitrate during vitamin A repletion were more pronounced than those observed during vitamin A depletion.

It is concluded from these data that dietary nitrate exerts its greatest influence on the vitamin A status of ruminants through a reduction in the amount of dietary vitamin A reaching hepatic stores rather than through an accelerated depletion of existing stores.

Table 1. Effect of 2.5% Sodium Nitrate on Lambs - Experiment 1 (136 days)

	Control	Vit. A	Carotene	Nitrate	Nitrate and Vit. A	Nitrate and Carotene
No. lambs	20	20	18	17	20	19
Init. wt.,lb.	69.5	69.5	68.6	70.0	69.7	69.7
Av. daily gain,lb.	0.394	0.367	0.418	0.339	0.370	0.361
Av. daily feed,lb.						
Concentrate mix	2.49	2.42	2.75	2.42	2.29	2.33
Supplement	0.25	0.25	0.25	0.25	0.25	0.25
Total	2.74	2.67	3.00	2.67	2.54	2.58
Feed/lb. gain,lb.	6.96	7.26	7.20	8.04	6.85	7.20
Av. plasma vit. A, mcg./100 ml.						
Initial	28	27	29	27	31	28
26 days	35			36		
46 days	36	35	36	37	43	35
67 days	37			37		
88 days	36	39	35	36	42	35
110 days	32			28		
132 days	27	36	37	19	41	31
Av. liver vit.A, mcg/gm.	9	22	14	3	18	9

Table 2. Effect of 2.5% Sodium Nitrate on Performance and Plasma Vitamin A of Lambs During Vitamin A Depletion - Experiment 2, Depletion Period (136 days).

	Control	Sodium Nitrate
Number lambs[a]	79	72
Init. wt.,lb.	69.7	70.2
Av. daily gain,lb.	0.387	0.348
Av. daily feed,lb.		
Concentrate mix	2.53	2.40
Supplement	0.25	0.25
Total	2.78	2.65
Feed/lb. gain,lb.	7.18	7.64
Av. plasma vit. A, mcg/100 ml.		
Initial	29	28
132 days	27	23

[a]Includes data from columns 1 and 4 in table 1.

Table 3. Effect of 2.5% Sodium Nitrate on Lambs During Vitamin A Repletion - Experiment 2, Repletion Period (43 days)

	Vit. A	Carotene	Nitrate & Vit. A	Nitrate & Carotene
Number of lambs	20	20	19	18
Init. wt.,lb.	121.2	123.6	116.8	118.8
Av. daily feed, lb.				
Concentrate mix	2.46	2.57	2.31	2.42
Supplement	0.50	0.50	0.50	0.50
Total	2.96	3.07	2.81	2.92
Feed/lb. gain,lb.	14.56	14.64	11.77	12.65
Av. plasma vit. A, mcg./100 ml.				
Initial	25	30	24	24
21 days	39	42	36	37
43 days	44	44	39	34
Av. Liver vit. A				
Mcg/gm.	25	18	10	7