

Influence of Triiodothyronine Injections on Calf Immune Response to an Infectious Bovine Rhinotracheitis Virus Challenge and Nitrogen Balance of Lambs^{1,2}

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ABSTRACT: Three experiments were conducted to determine the influence of triiodothyronine (T₃) or propylthiouracil (PTU) on the humoral immune response of calves challenged with infectious bovine rhinotracheitis virus (IBRV) and on nitrogen depletion and repletion of lambs deprived of feed and water for 3 d. In Exp. 1, 18 steer calves (BW 284 ± 6 kg) challenged with IBRV were limit-fed (1.5% BW) a 60% concentrate diet and injected (s.c.) daily with alkaline saline, 4 mg of T₃, or .8 mg of T₃. Injections of T₃ did not affect serum antibody titers to IBRV, blood leukocyte counts, or plasma free fatty acid, ceruloplasmin, and cholesterol concentrations but increased ($P < .05$) plasma glucose concentrations and decreased ($P < .05$) plasma urea N concentrations. In Exp. 2, 36 IBRV-challenged steers (BW 266 ± 8 kg) were given ad libitum access to a 60% concentrate diet and injected (s.c.) daily with alkaline saline, .2 mg of T₃, or .4 mg of T₃. In contrast to Exp. 1, injections of T₃ did not affect plasma glucose or urea N concentra-

tions and reduced ($P < .05$) serum antibody titers to IBRV. In Exp. 3, eight wether lambs were limit-fed (600 g/d) a 36% concentrate pelleted diet and assigned to one of four treatments in a replicated 4 × 4 Latin square designed nutrient balance experiment involving periods of nutrient depletion and repletion. Treatments were as follows: 1) alkaline saline injection (s.c.), 2) 4 mg of PTU/kg BW in water, 3) .15 mg of T₃ s.c. daily for 15 d, and 4) .15 mg of T₃ s.c. daily for 7 d after the 3-d feed and water deprivation period. Thyroid status affected ($P < .05$) predeprivation N balance but did not affect N losses during the feed and water deprivation period. Retention of N during realimentation was affected ($P < .05$) by T₃ treatment. Results of these experiments indicate that there is a complex interrelationship among stress, nutrient status, and thyroidal status and that the effects of T₃ injections on immune and metabolic responses may be dependent on the nutritional status of the animal.

Key Words: Triiodothyronine, Steers, Lambs, Immunity, Stress

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Introduction

Bovine respiratory disease is the most economically costly disease of the U.S. beef cattle industry. This disease complex is caused by a combination of immunosuppressing stressors, viral infection(s), and bacterial infection(s) (Hoerlein and Marsh, 1957). The immunosuppressing effects of stressors have routinely been blamed on the increased secretion of glucocorticoids; however, it is now apparent that other

hormones may also be involved (Fabris, 1973; Gala, 1991).

Blood concentrations of triiodothyronine (T₃) decline in response to stressors such as feed and water restriction (Blum and Kunz, 1981), protein deficiency (Ash et al., 1985), heat (Christopherson et al., 1979), parasitic infection (Ash et al., 1985), and following dexamethasone injections (Cavalieri et al., 1984), types of stressors that normally occur during the marketing and transport of feeder calves from the farm of origin to commercial feedyards or stocker operations. However, the effects of these changes in T₃ concentrations on immune response and nutrient metabolism of feeder calves are not known.

These experiments were conducted to determine whether thyroid status could improve the humoral immune response of calves to a viral infection (infectious bovine rhinotracheitis virus; IBRV) and whether it would affect N losses during a feed and water deprivation period in lambs.

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tested by comparing daily means to d-0 means using Dunnett's two-tailed test. If a treatment × day interaction was obtained ($P < .10$), treatment effects were tested by analyzing the data within day as a randomized block design with treatment effects tested by linear and quadratic contrasts, and day of infection effects were compared by analyzing the data within treatments by ANOVA and comparing means to d-0 means using Dunnett's two-tailed test. Data for the 6-h postinjection portion of the experiment were analyzed by ANOVA as a split-plot in time with sampling time as the subplot. Serum antibody titers were converted to log base 2 for statistical analysis.

Experiment 2. Thirty-six feeder steer calves of British breeding averaging 266 ± 8 kg were randomized to one of three treatments. Results of Exp. 1 suggested that the .8 mg/d dosage of T₃ may have been excessive; therefore, in Exp. 2 the T₃ dosages were reduced. Treatments were daily s.c. injections of 1) alkaline saline (Control), 2) .2 mg of T₃ in alkaline saline, and 3) .4 mg of T₃ in alkaline saline starting on d -4 and continuing through d 10 (d 0 = IBRV challenge). Calves were housed in three pens equipped with automatic feeders (Pinpointer 4000B, UIS Corp., Cookeville, TN) to determine individual daily feed intakes. An equal number of calves from each treatment were assigned to each pen. Calves were given ad libitum access to a 60% concentrate diet (Table 1) and water throughout the experiment.

Following a 28-d adaptation period and 4 d of T₃ injections, calves were challenged with IBRV as in Exp. 1. Individual daily feed intakes were recorded for d -4 to 21. Blood samples were obtained via jugular venipuncture on d 0, 7, 10, 14, 21, and 28. All other procedures were the same as in Exp. 1.

Data were analyzed by ANOVA as a split-plot in time. Treatment effects were tested by linear and quadratic contrasts using animal within treatment as the error term. Day effects were tested by comparing daily means to means on d 0 by Dunnett's two-tailed test.

Experiment 3. Eight Hampshire wether lambs (average BW 35 ± 3 kg) were used in a replicated 4 × 4 Latin square designed N balance experiment. Each period of the Latin square lasted 8 wk and consisted of 1) a 7-d digestion stall adjustment period, 2) a 4-d predeprivation period, 3) a 3-d feed and water deprivation period, 4) a 7-d realimentation period, and 5) a 35-d rest period out of digestion stalls. During the rest period, lambs were individually housed in 1.5-m × 2-m pens with slotted floors. During fecal and urine collections, lambs were confined to stainless steel digestion stalls (102 cm × 64 cm). Temperature in the metabolism facility was maintained at 18°C, and humidity was maintained at 30 to 50%. Lambs were provided continual access to deionized water and were limit-fed (600 g/d, as-fed basis) a 36% concentrate pelleted diet (Table 1) except during the deprivation period.

Lambs were assigned to one of four treatments during each experimental period: 1) control, 2) hypothyroid, 3) hyperthyroid, and 4) realimentation hyperthyroid. Control lambs were injected s.c. each day with 1 mL of alkaline saline (pH 8.5) beginning 5 d before the start of the deprivation period and continuing through the deprivation and realimentation periods. To effect a hypothyroid-like condition, lambs were given 4 mg/kg BW of the thyroid depressant propylthiouracil (PTU; Sigma Chemical, St. Louis, MO) in their drinking water each day starting 29 d before the feed and water deprivation period (during the rest period out of digestion stalls) and continuing through the realimentation period. A solution (4 mL/kg BW) containing 1 g of PTU/L was provided as the lambs' only water immediately after feeding each day to assure that lambs received the proper dosage of PTU. Hyperthyroid lambs were injected s.c. with .15 mg of T₃ in alkaline saline on the same days control lambs were injected. Realimentation hyperthyroid lambs were injected s.c. with alkaline saline during the predeprivation and feed and water deprivation periods and injected s.c. with .15 mg of T₃ in alkaline saline during the 7-d realimentation period. Results of previous studies (Cooper et al., 1983) and Exp. 1 and 2 suggested that the length of time between sample collections in the Latin square (6 wk) was sufficient to prevent treatment carryover effects. Cooper et al. (1983) noted that serum T₃, T₄, and PTU and intrathyroidal PTU concentrations returned to baseline values within 10 d after discontinuation of PTU administration when rats were provided PTU in their drinking water for 30 d. Initial analysis of Exp. 1 and 2 indicated that serum T₃ and T₄ concentrations returned to baseline values within 14 d of discontinuation of T₃ injections.

Total feces and urine excreted were collected, weighed, and subsampled daily during the predeprivation, deprivation, and realimentation periods. Subsamples were composited for the 4-d predeprivation period, the 3-d deprivation period, and the 7-d realimentation period. Urine samples were stored at -20°C for later analysis. A portion of the fecal sample was stored at -20°C for N analysis, and a second portion was dried to a constant weight at 60°C and ground through a 1-mm screen in a Wiley mill. Feed samples were collected daily, dried, and ground. Following block digestion, feed, fecal, and urine samples were analyzed for concentrations of N by automated analysis (Technicon, 1977).

Blood samples were collected by jugular venipuncture before the morning feeding at the start of the collection period, start of the deprivation period, end of the deprivation period, and end of the realimentation period. Blood was allowed to clot at room temperature for 90 min, centrifuged, and the serum decanted. Serum was analyzed for concentrations of urea N, cholesterol, T₄, T₃, Cu, Zn, and Fe as previously described.

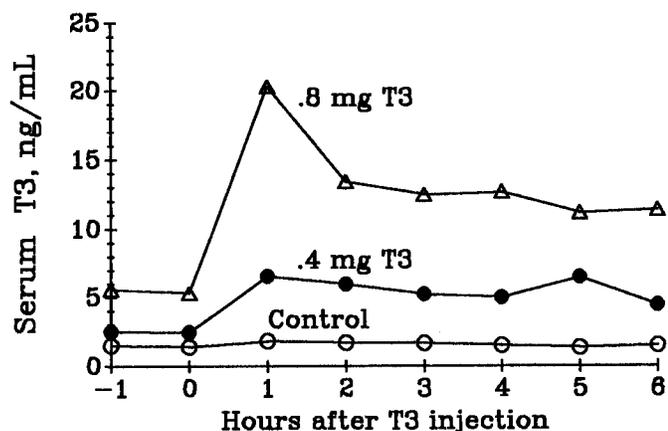


Figure 1. Serum triiodothyronine (T₃) concentrations of calves for 6 h following s.c. injections of alkaline saline (Control: open circles), .4 mg of T₃ (closed circles), and .8 mg of T₃ (triangles) in Exp. 1. The pooled SEM was .82 ng/mL. Serum concentrations of T₃ increased linearly ($P < .05$) with increasing T₃ dosage at all sampling times.

Nitrogen balance data were statistically analyzed within sampling periods by ANOVA as a replicated 4 × 4 Latin square using the GLM procedures of SAS (1988). Treatment effects were compared by Waller-Duncan multiple range test if a significant F -test was obtained. Serum data were analyzed by ANOVA as a split-plot with the main plot in a replicated 4 × 4 Latin square design. Sampling period was the subplot. Treatment effects were compared by Waller-Duncan multiple range test if a significant F -test was obtained. Sample period effects were tested by comparing period means to the mean for the predeprivation period by Dunnett's test. If a significant treatment × sample day interaction was obtained, data were subsequently analyzed by ANOVA within sample period as a replicated 4 × 4 Latin square with treatment effects compared by Waller-Duncan multiple range test. To test sample period effects, data were also analyzed within treatment by ANOVA as a completely randomized design and period means were compared to predeprivation means using Dunnett's test.

Results

Experiment 1. Over the 6-h postinjection period, serum concentrations of T₃ increased (linear effect, $P < .05$) with increasing dosage of T₃ (Figure 1). Concentrations of T₃ increased rapidly after T₃ injections but tended to be constant over the remainder of the 6-h sampling period. Serum concentrations of T₄ were relatively constant over the sampling period, although calves injected with T₃ had lower (P

Table 2. Serum triiodothyronine (T₃) and thyroxine (T₄) concentrations of steer calves before and after intranasal challenge with infectious bovine rhinotracheitis virus (IBRV) in Exp. 1 and 2

Time	Control	Low T ₃ ^a	High T ₃ ^a	SEM
T ₃ , ng/mL				
Day -4	1.94	1.45 ^c	1.36 ^c	.13
Day 0 ^b	2.08	2.14	3.56	.20
Day 3 ^b	1.61	2.38	3.82	.27
Day 5 ^b	1.17 ^c	1.58 ^c	3.27	.27
Day 7 ^b	1.38 ^c	2.18	4.52	.38
Day 10 ^b	2.04	2.46	3.61	.30
Day 14 ^b	2.11	1.76	.89 ^c	.18
Day 21	1.57	1.53 ^c	1.06 ^c	.16
T ₄ , ng/mL				
Day -4	62.7	41.7 ^d	50.8 ^d	3.8
Day 0 ^b	74.2	21.0	20.2	6.4
Day 3 ^b	54.2 ^d	17.0	17.5	5.8
Day 5 ^b	59.5 ^d	10.8	6.8	6.3
Day 7 ^b	57.0 ^d	22.5	6.5	6.9
Day 10 ^b	100.8 ^c	18.0	9.0	12.1
Day 14 ^b	84.7	38.0	18.5	7.7
Day 21	68.5	56.8 ^d	54.3 ^d	4.3
Exp. 2				
T ₃ , ng/mL				
Day 0	1.81	1.37	2.38	.12
Day 7	1.13 ^d	.98 ^d	1.26 ^d	.08
Day 10	1.65	1.79	1.43 ^d	.10
Day 14	1.51	1.80	1.64	.10
Day 21	1.75	1.62	1.64	.09
Day 28	1.88	1.60	1.76	.10
T ₄ , ng/mL				
Day 0 ^b	95.8	48.3	33.2	6.1
Day 7 ^b	66.7 ^d	28.4 ^d	7.8 ^d	5.1
Day 10 ^b	115.8	66.2	10.4 ^d	9.5
Day 14 ^b	108.3	104.0	76.3 ^d	6.4
Day 21	104.2	85.0	95.0 ^d	4.9
Day 28	91.7	88.0	82.1 ^d	4.8

^aLow T₃ received .4 mg of T₃/d in Exp. 1 and .2 mg of T₃/d in Exp. 2. High T₃ received .8 mg of T₃/d in Exp. 1 and .4 mg of T₃/d in Exp. 2. Control calves received alkaline saline injections. Injections started on d -4 and continued through d 10. Calves were challenged with IBRV on d 0.

^bLinear effect of T₃ injections ($P < .01$).

^cDifferent from d 0 ($P < .05$).

^dDifferent from d 0 ($P < .10$).

$< .05$) serum T₄ concentrations than control calves (mean 32 vs 65 mg/mL, respectively). Rectal temperature and BW over the 6-h postinjection period were not significantly affected by T₃ injections (means 39.1 ± .04°C and 284.3 ± 2.3 kg, respectively).

Concentrations of T₃ and T₄ in serum samples obtained on d -4 to 21 were affected by both T₃ injections and IBRV infection (Table 2). In control steers, T₃ concentrations declined ($P < .05$) during IBRV infection but returned to prechallenge concentrations by d 10 postchallenge. Serum T₃ concentrations increased linearly ($P < .01$) as T₃ dosage increased. Serum T₄ concentrations of control calves tended ($P < .10$) to decline during IBRV infection but

Table 3. Serum antibody titers^a to infectious bovine rhinotracheitis virus (IBRV) in calves inoculated with IBRV in Exp. 1 and 2

Time	Control	Low T ₃ ^b	High T ₃ ^b	SEM
Exp. 1				
Day 0	.00	.00	.00	—
Day 7	.00	1.50	.50	.34
Day 10 ^c	.50	3.17	1.00	.57
Day 14 ^c	3.17	4.67	3.83	.41
Day 21 ^c	3.67	4.83	3.83	.37
Day 28 ^c	3.67	5.00	3.83	.35
Exp. 2				
Day 0	.00	.00	.00	—
Day 7	.08	.20	.00	.07
Day 10	1.17	.80	.00	.30
Day 14 ^d	4.08	2.70	2.50	.24
Day 21 ^d	4.58	3.30	3.20	.22

^aLog base 2.

^bLow T₃ received .4 mg of T₃/d in Exp. 1 and .2 mg of T₃/d in Exp. 2. High T₃ received .8 mg of T₃/d in Exp. 1 and .4 mg of T₃/d in Exp. 2. Injections started on d -4. Calves were challenged with IBRV on d 0.

^cQuadratic effect of T₃ injections ($P < .15$).

^dLinear effect of T₃ injections ($P < .05$).

returned to values higher ($P < .05$) than prechallenge values on d 10 postchallenge. Serum T₄ concentrations decreased linearly ($P < .01$) with increasing T₃ dosage. Serum T₄ concentrations of calves injected with T₃ were still decreased 4 d after T₃ injections were stopped (d 14) but had returned to preinjection concentrations by d 21.

Serum antibody titers to IBRV were not significantly affected by T₃ injections, although on d 10, 14,

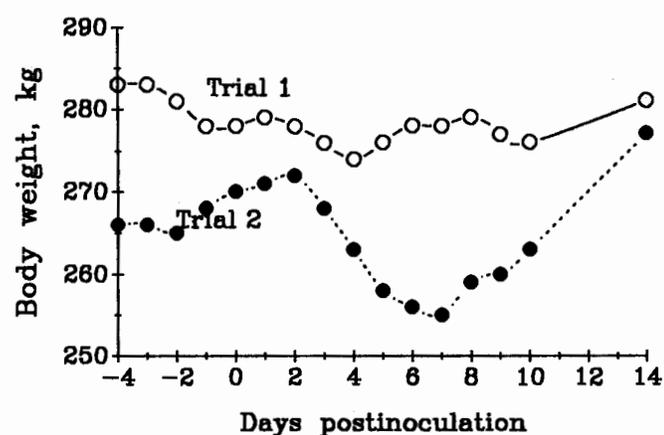


Figure 2. Mean BW of calves before and after intranasal inoculation with infectious bovine rhinotracheitis virus in Exp. 1 (open circles) and 2 (closed circles). Calves were inoculated on d 0. The pooled SEM for Exp. 1 was 1.4 kg and for Exp. 2 was 1.0 kg. In Trial 2, mean BW on d 5 to 9 after inoculation were lower ($P < .05$) than on d 0.

21, and 28 postchallenge, calves injected with .4 mg of T₃/d tended to have higher (quadratic effect, $P < .15$) antibody titers than calves injected with alkaline saline or .8 mg of T₃ (Table 3). Calf BW and rectal temperatures were not affected ($P > .20$) by T₃ injections. Calves lost little weight during the IBRV infection, probably because feed intake was limited and there were no feed refusals during the IBRV infection (Figure 2). Rectal temperatures were elevated ($P < .05$) by d 3 postchallenge and remained elevated through d 6 (Figure 3).

Total leukocyte, lymphocyte, and eosinophil counts were not affected ($P > .20$) by T₃ injections or by IBRV infection ($P = .12$). Total leukocyte counts were $9,748 \pm 488/\mu\text{L}$ on d 0 and $7,930 \pm 460/\mu\text{L}$ on d 3 postchallenge. Lymphocyte counts were $6,009 \pm 334/\mu\text{L}$ on d 0 and $4,706 \pm 245/\mu\text{L}$ on d 7 postchallenge. Eosinophil counts were $398 \pm 143/\mu\text{L}$ on d 0 and $76 \pm 18/\mu\text{L}$ on d 7 postchallenge.

Serum concentrations of Cu, Zn, Fe, and ceruloplasmin and TIBC were not affected ($P > .20$) by T₃ injections but were affected by IBRV infection (Table 4). Mean serum Cu and ceruloplasmin concentrations increased ($P < .08$) during IBRV infection and returned to prechallenge concentrations by d 14 postchallenge. Serum Zn and Fe concentrations declined ($P < .10$) during the IBRV infection. Serum TIBC did not significantly change during the first 3 d after the challenge then decreased ($P < .08$) on d 7 postchallenge.

During IBRV infection, serum glucose and cholesterol concentrations decreased ($P < .05$), whereas plasma urea N concentrations increased ($P < .05$; Table 5). Calves injected with T₃ had higher

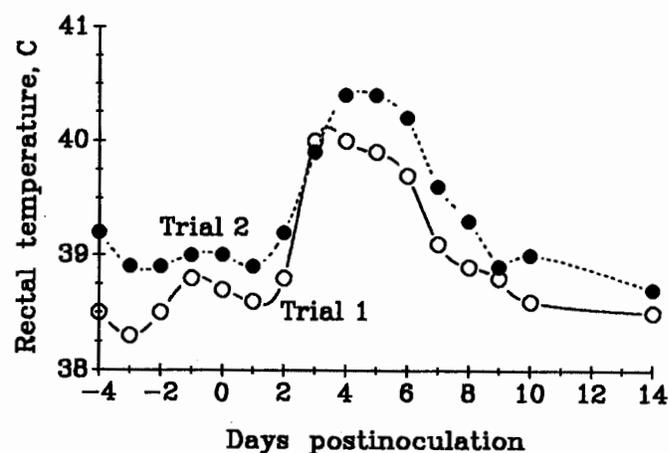


Figure 3. Mean rectal temperature of calves before and after intranasal inoculation with infectious bovine rhinotracheitis virus in Exp. 1 (open circles) and 2 (closed circles). Calves were inoculated on d 0. The pooled SEM for Exp. 1 was .13°C and for Exp. 2 was .06°C. In both trials, mean rectal temperatures on d 3 to 7 after inoculation were greater ($P < .05$) than on d 0.

Table 4. Mean serum Cu, ceruloplasmin, Zn, Fe, and total-iron-binding capacity (TIBC) of calves challenged with infectious bovine rhinotracheitis virus (IBRV) in Exp. 1 and 2

Time	Copper, μg/mL	Cerulo- plasmin, μmol·m ⁻¹ ·L ⁻¹	Zinc, μg/mL	Iron, μg/mL	TIBC, μg/100 mL
Day -4 ^a	.76	127	1.08	121	344
Day -1	.72	129	1.26	138	349
Day 0	.69	138	1.22	95	353
Day 3	.88 ^b	142	1.14	87	390
Day 5	1.02 ^b	156 ^b	.97 ^c	52 ^c	352
Day 7	.73	159 ^b	1.01 ^c	62 ^c	312 ^c
Day 10	.67	157 ^b	1.16	110	380
Day 14	.69	144	1.08	128	350
Day 21	.76	148	1.02 ^c	111	355
SEM	.02	3	.02	3.4	4
Exp. 2					
Day 0	1.06	118	1.30	144	452
Day 7	1.13	118	1.04 ^b	57 ^b	356 ^b
Day 10	1.00	113	1.02 ^b	73 ^b	392 ^b
Day 14	1.03	109	1.12	106 ^b	369 ^b
Day 21	1.09	116	1.10 ^b	120	413
Day 28	.97	109	1.08 ^b	123	401
SEM	.03	4	.03	5.6	9

^aInjections of T₃ began on d -4 and ended on d 10. Calves were challenged with IBRV on d 0.

^bDifferent from d 0 ($P < .05$).

^cDifferent from d 0 ($P < .10$).

(linear effect, $P < .05$) serum glucose concentrations and tended to have lower (linear effect, $P < .09$) urea N concentrations than control calves. Serum FFA concentrations were not affected ($P > .20$) by IBRV infection or T₃ injections (mean 482 ± 53 μmol/L).

Experiment 2. In all treatment groups, serum T₃ and T₄ concentrations tended to decline ($P < .10$) between d 0 and 7 of the IBRV challenge (Table 2). Serum T₃ concentrations were not affected ($P > .20$) by T₃ injections; however, serum T₄ concentrations were reduced by T₃ injections (linear effect; $P < .01$).

Body weight, rectal temperature, and DMI were not affected ($P > .20$) by T₃ injections in Exp. 2. During IBRV infection, BW (Figure 2) and DMI (Figure 4) decreased ($P < .05$) and rectal temperature (Figure 3) increased ($P < .05$).

In contrast to Exp. 1, calves injected with T₃ had lower (linear effect, $P < .05$) serum antibody titers to IBRV than control calves on d 14 and 21 after the challenge (Table 3).

Serum concentrations of Cu, Zn, Fe, and ceruloplasmin and TIBC were not significantly affected by T₃ injections in Exp. 2 (Table 4). Serum concentrations of Zn and Fe and TIBC declined ($P < .05$) during IBRV infection. Serum Cu and ceruloplasmin concentrations were not affected by IBRV infection in Exp. 2, possibly as a result of sampling intervals.

As in Exp. 1, serum cholesterol concentrations declined ($P < .05$) during IBRV infection (Table 5). Injections of T₃ did not significantly affect serum

cholesterol. Plasma glucose and urea N concentrations were not affected by IBRV infection or T₃ injections in Exp. 2 (means 89.9 ± 1.2 and $7.62 \pm .14$ mg/100 mL, respectively).

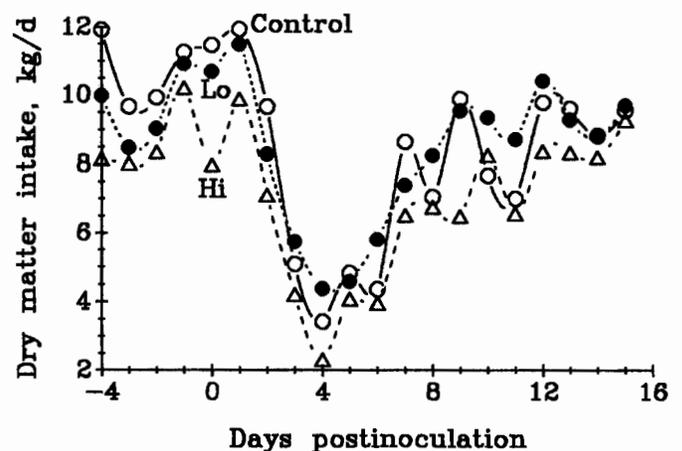


Figure 4. Daily dry matter intakes of calves before and after intranasal inoculation with infectious bovine rhinotracheitis virus in Exp. 2. Calves were inoculated on d 0. Control = open circles, .2 mg of triiodothyronine/d = closed circles, .4 mg of triiodothyronine/d = triangle. The pooled SEM was .14 kg/d. Mean DMI on d 3 to 7 after inoculation were lower ($P < .05$) than on d 0.

Table 5. Blood metabolite concentrations of calves challenged with infectious bovine rhinotracheitis virus (IBRV) in Exp. 1 and 2

Time	Control	Low T ₃ ^a	High T ₃ ^a	SEM
Glucose, mg/100 mL				
Day -4	86	79	81	4.9
Day 0 ^b	87	107	99	3.2
Day 3 ^b	88	115 ^c	98	2.7
Day 5 ^b	81 ^c	99 ^c	98	2.6
Day 7 ^b	82 ^c	94 ^c	95	2.6
Day 10 ^b	80 ^c	104	104	4.1
Day 14/21	81 ^c	91 ^c	78 ^c	2.3
Cholesterol, mg/100 mL				
Day -4	111	122	118	6.7
Day 0	102	114	103	5.4
Day 3	97	108	94	5.2
Day 5	87 ^c	102	86 ^c	5.8
Day 7	80 ^c	82 ^c	74 ^c	5.1
Day 10	83 ^c	86 ^c	71 ^c	4.2
Day 14/21	80 ^c	93 ^c	84 ^c	3.2
Urea N, mg/100 mL				
Day -4	6.42	7.07	6.32	.37
Day 0 ^b	7.28	5.80	5.58	.37
Day 3 ^b	8.00	6.30	6.07	.42
Day 5 ^b	8.55 ^c	7.38 ^c	7.87 ^c	.53
Day 7 ^b	9.13 ^c	7.23 ^c	8.35 ^c	.50
Day 10	6.63	6.53	6.30	.33
Day 14/21	5.68	5.59	5.38	.27
Exp. 2				
Cholesterol, mg/100 mL				
Day 0	96	83	89	4.3
Day 7	91 ^c	80	80 ^c	3.5
Day 10	80 ^c	73 ^c	75 ^c	2.8
Day 14	78 ^c	75 ^c	82	3.5
Day 21/28	95	97	96	3.6

^aIn Exp. 1, Low T₃ received .4 mg of T₃/d; High T₃ received .8 mg of T₃/d. In Exp. 2, Low T₃ received .2 mg of T₃/d and High T₃ received .4 mg of T₃/d. Injections of T₃ began on d -4. Calves were challenged with IBRV on d 0.

^bLinear effect of T₃ injections ($P < .05$).

^cDifferent from d 0 ($P < .05$).

Experiment 3. As in Exp. 1, compared with controls, injections of T₃ increased ($P < .05$) serum T₃ concentrations and decreased ($P < .05$) serum T₄ concentrations during the predeprivation period (Table 6). Compared with control lambs, providing PTU in the drinking water did not cause a significant reduction in circulating T₃ concentrations but reduced ($P < .08$) circulating T₄ concentrations during the predeprivation period.

Compared with predeprivation values, feed and water deprivation caused a decrease ($P < .06$) in serum T₃ concentrations in lambs on the control and T₃ treatments but not in lambs on the PTU treatment. Feed and water deprivation also caused a reduction ($P < .06$) in serum T₄ concentrations of control lambs but had no effect when lambs were given PTU or T₃. At the end of the feed and water deprivation period, serum T₃ concentrations were similar for all treatment groups, and T₄ concentrations were lower ($P < .05$) in lambs injected with T₃ than in control and PTU-treatment lambs. At the end of the realimentation period no differences were observed among treatments for serum T₃ concentrations; however, control and PTU-treated lambs had higher ($P < .01$) T₄ concentrations than lambs receiving T₃ injections.

During the predeprivation period, retention of N was greater ($P < .05$) in lambs given PTU than in lambs injected with T₃. Control lambs were intermediate (Table 7). Nitrogen losses during the deprivation period were not affected ($P > .20$) by treatment. During the realimentation period, lambs that received T₃ injections during the predeprivation, deprivation, and realimentation periods had greater ($P < .05$) N balance than lambs that received T₃ injections only during the realimentation period. Control and PTU-treated lambs were intermediate. This difference was due to a combination of greater ($P < .05$) urinary N and fecal N losses in lambs receiving T₃ injections only

Table 6. Serum triiodothyronine (T₃) and thyroxine (T₄) concentrations of lambs in Exp. 3

Item/time	PTU ^a	Control ^a	T ₃ R ^a	T ₃ ^a	SEM
T ₃ , ng/mL					
Pre-T ₃ ^b	1.64	1.72	1.76	1.73	.15
Predepr. ^b	1.41 ^c	1.84 ^c	1.80 ^c	2.51 ^d	.26
End depr. ^b	1.21	1.20 ^f	1.24 ^f	1.22 ^f	.15
Fed 7 d	2.00	1.81	1.64	1.53	.13
T ₄ , ng/mL					
Pre-T ₃	50.0	61.0	61.8	51.8	5.0
Predepr.	40.8 ^d	66.9 ^e	56.5 ^{de}	18.5 ^c	5.5
End depr.	43.6 ^c	52.2 ^{cf}	47.6 ^c	21.5 ^d	4.3
Fed 7 d	63.8 ^c	57.3 ^c	17.1 ^d	14.0 ^d	5.5

^aPTU = lambs given 4 mg of propylthiouracil/kg BW each day beginning 28 d before the feed and water deprivation period; Control = daily alkaline saline injection, T₃R = .15 mg of T₃ daily during the realimentation period only; T₃ = .15 mg of T₃ daily beginning 5 d before the deprivation period.

^bPre-T₃ = sample obtained 5 d before the start of the deprivation period and before first injection of T₃ to lambs on treatment T₃. Predepr. = start of deprivation period; End depr. = end of deprivation period.

^{c,d,e}Means within a row lacking a common superscript differ ($P < .05$).

^fDifferent from predeprivation mean ($P < .06$).

Table 7. Predeprivation, deprivation, and realimentation nitrogen metabolism of lambs in Exp. 3

Period/item	PTU ^a	Control ^a	T ₃ R ^a	T ₃ ^a	SEM
Predeprivation ^b					
Intake, g	43.7	43.7	43.7	43.7	.73
Urine, g	17.5	18.8	19.5	19.4	.86
Fecal, g	21.8	22.7	21.2	23.4	.49
Balance, g	4.4 ^c	2.1 ^{cd}	3.0 ^{cd}	.8 ^d	1.35
Deprivation ^b					
Urine, g	17.7	18.1	18.0	17.5	.36
Fecal, g	7.0	7.2	7.8	7.3	.20
Balance, g	-24.7	-25.3	-25.7	-24.8	.46
Realimentation					
Intake, g	73.4	76.2	76.2	72.4	1.45
Urine, g	26.4 ^c	26.8 ^c	26.7 ^c	21.9 ^d	1.39
Fecal, g	33.6 ^c	33.4 ^c	38.0 ^d	33.2 ^c	.87
Balance, g	13.4 ^{cd}	16.0 ^{cd}	11.6 ^c	17.3 ^d	1.94
Cumulative balance, g	-6.9	-7.2	-11.1	-6.7	3.09

^aPTU = lambs received 4 mg of propylthiouracil/kg BW daily; Control = lambs injected daily with alkaline saline; T₃R = lambs received .15 mg of T₃/d during realimentation period; T₃ = lambs received .15 mg of T₃/d during the entire trial.

^bDuring the predeprivation and deprivation periods Control and T₃R lambs received the same treatment.

^{c,d}Means within a row lacking a common superscript differ ($P < .05$).

during the realimentation period. Cumulative N retention over the 14-d experimental period was not affected by thyroid status.

Plasma urea N concentrations increased ($P < .01$; 10.3 vs 20.5 mg/100 mL for pre- and postdeprivation, respectively) during feed and water deprivation but were not affected by treatment. Serum cholesterol (mean 70.8 ± 2.1 mg/100 mL), Cu (mean $1.0 \pm .08$ μ g/mL), and Zn (mean $1.2 \pm .11$ μ g/mL) were not affected by feed and water deprivation or thyroid status. Serum concentrations of Fe declined (86 vs 75 μ g/mL; $P < .09$) during feed and water deprivation but were not affected by thyroid treatment.

Discussion

In Exp. 1 and 2, serum T₃ and T₄ concentrations declined ($P < .10$) during IBRV infection, and the changes tended to coincide with changes in rectal temperature. In Exp. 3, feed and water deprivation also reduced serum T₃ and T₄ concentrations. In Exp. 1, when feed intake was restricted, DMI remained relatively constant during the IBRV infection, whereas in Exp. 2 when feed was available ad libitum, DMI decreased ($P < .01$) during infection. The decreases in serum T₃ and T₄ concentrations noted in Exp. 2 were similar in magnitude (38% and 30%, respectively) to those seen in Exp. 1 and 3, suggesting that the decreases in serum T₃ and T₄ concentrations caused by infection and/or feed deprivation are not additive.

In all three experiments, injections of T₃ reduced ($P < .05$) serum T₄ concentrations, although in Exp. 2 and 3, serum concentrations of T₃ were not markedly

affected by T₃ injections. This may have been due to the dosage of T₃ and/or to the sampling intervals used.

Thyroidal status is regulated by at least two factors: 1) secretion of T₄ through hypothalamo-hypophyseal control and 2) peripheral deiodination of T₄ to T₃ and its interactions with probably nuclear receptor sites. It seems that energy availability (Eales, 1988), and possibly protein status (Hammond et al., 1984), are major factors regulating peripheral thyroid status in ruminants. In most mammals it seems that nutrient intake has a greater effect on peripheral conversion of T₄ to T₃ than on T₄ secretion (Eales, 1988). In humans, Aun et al. (1983) noted that there was an increased conversion of T₄ to reverse-T₃ with a proportional decrease in circulating T₃ concentrations during systemic disease, after surgical trauma, during food deprivation, during corticosteroid treatment, and following catecholamine injections. Hasselgren et al. (1987) also noted that muscle concentrations of T₃ increased during sepsis and suggested that the increased uptake of T₃ by muscle was partially responsible for the increased muscle proteolysis noted during infection. These results suggest that the dosage of T₃ required to maintain "normal" circulating T₃ concentrations may be dependent on both the nutritional and/or stress status of the animal.

The changes in BW and DMI noted during IBRV infection in Exp. 1 and 2 were typical of those seen in previous IBRV challenge studies (Cole et al., 1986; Orr et al., 1988, 1990; Chirase et al., 1991). The postchallenge decline in BW was greater in Exp. 2 than in Exp. 1, probably because of a loss of gut fill caused by the decrease ($P < .01$) in DMI in Exp. 2.

The changes in rectal temperature, antibody titers, and blood leukocyte counts noted during IBRV infection in Exp. 1 and 2 were also typical of those seen in previous studies (Cole et al., 1986; Orr et al., 1988, 1990; Chirase et al., 1991). With restricted intakes in Exp. 1, injections of T₃ did not significantly affect antibody titers to IBRV, although there was a trend ($P = .14$) for increased antibody response in calves injected with .4 mg of T₃/d. In contrast, with ad libitum intake in Exp. 2, injections of T₃ (.4 mg/d) reduced ($P < .05$) antibody response. These results are not consistent with those of Filteau et al. (1987a,b) and Filteau and Woodward (1987), who reported an increased antibody response to sheep red blood cells in malnourished mice given oral T₃ but noted no improvement when mice were well-nourished. Calves in Exp. 1 were restricted to nutrient intakes slightly above maintenance. It is possible that a more severe nutritional deficiency may be required for T₃ to elicit an increased antibody response in ruminants. These results and those of Fabris (1973) suggest that the effects of T₃ injections on humoral immune response may depend on both the thyroid status and nutritional status of the animal as well as the dosage of T₃ used.

Comsa et al. (1979) noted that severe hypothyroidism caused a reduction in circulating concentrations of lymphocytes. In Exp. 1, IBRV infection caused both a mild hypothyroidism and a modest reduction in circulating lymphocyte concentrations. Although the decline in serum T₃ concentrations was prevented with supplemental T₃, the decline in lymphocyte counts was not prevented by T₃ injections.

The changes in serum minerals and metabolites noted during IBRV infection in Exp. 1 and 2 tend to agree with results of other studies (Cole et al., 1986; Orr et al., 1988, 1990; Chirase et al., 1991). Although not tested statistically, changes in serum Cu, Zn, ceruloplasmin, and Fe concentrations seemed to be positively related to changes in rectal temperature, whereas the changes in TIBC seemed to be inversely related to rectal temperature. Plasma glucose concentrations decreased ($P < .05$) and urea N concentrations increased ($P < .05$) during IBRV infection when feed intake was limited (Exp. 1) but were not affected by IBRV infection when calves had ad libitum access to feed (Exp. 2). These differences may be due to the availability of nutrients within the digestive tract during the infection period. Serum cholesterol concentrations were not affected by T₃ injections but declined ($P < .05$) during IBRV infection in both trials. The cause for the reduced serum cholesterol concentrations during infection is not apparent. O'Kelly (1986) noted a decrease in serum cholesterol in cattle during heat stress that paralleled the increased quantities of fatty acids lost through the feces.

Declines in serum Zn, Fe, and glucose with concomitant increases in serum Cu, ceruloplasmin,

and urea N are typical of the acute-phase response that occurs in the early stages of infection (Grimble, 1990). Injections of T₃ had consistent effects across experiments on measures of the acute phase response. Injections of T₃ did not affect rectal temperature, BW change, DMI, or serum concentrations of Zn, Fe, Cu, and ceruloplasmin. Although injections of T₃ did not prevent a decline in plasma glucose concentrations during IBRV infection, in Exp. 1, injections of T₃ increased plasma glucose concentrations before the challenge such that plasma glucose concentrations during the IBRV infection were higher ($P < .05$) in calves treated with T₃.

The physiological changes that occur during the acute-phase response are part of a cascade of events involving several cytokines, many of which are still not clearly understood (Grimble, 1990). Some of these physiological changes are considered to be potentially beneficial to the host (lower serum Fe and Zn, febrile response), whereas others are considered to be of questionable benefit or even detrimental to the host (hypoglycemia, tumor-necrosis-factor secretion). Results of these studies suggest that injections of T₃ do not have adverse effects on the acute-phase response.

It was anticipated that T₃ injections might increase N losses in Exp. 3 because, in humans on a low-calorie diet, treatment with T₃ increased urinary N loss (Carter et al., 1975; Koppeschaar et al., 1983) and, in lambs, injections of T₃ caused a decrease in diet DM digestibility via an increased rate of passage through the gut (Kennedy et al., 1977). However, in the present experiment, thyroidal status seemed to have variable effects on N metabolism. This variability may have been due in part to the relatively minor effects that PTU feeding and T₃ injections had on plasma T₃ and T₄ concentrations. Providing lambs 4 mg of PTU/kg BW in their drinking water tended ($P < .08$) to reduce serum T₄ concentrations in Exp. 3 but had no significant effect on circulating T₃ concentrations. In contrast, Rumsey et al. (1985) reported that steers fed 4 mg of PTU/kg BW had lowered serum concentrations of both T₃ and T₄. At lower PTU dosages, T₃ concentrations were decreased but T₄ concentrations were increased, suggesting that high dosages of PTU had a direct effect on thyroid activity (i.e., secretion of T₄) but lower dosages primarily affected hepatic and renal deiodination of T₄ to T₃. In vitro studies (Kahl et al., 1985) confirmed these results.

During the predeprivation period, lambs given PTU had greater ($P < .05$) N balance than lambs injected with T₃. During the realimentation period, PTU had no effect on N balance; however, the effects of T₃ injection on N balance seemed to be related to the length of time lambs had been on the T₃ treatments. During the realimentation period, lambs that received T₃ injections only during the realimentation period (realimentation hyperthyroid lambs) had greater

urinary and fecal N losses and lower N balance than lambs given T₃ during the predeprivation, deprivation, and realimentation periods. The greater urinary and fecal N losses in these lambs is consistent with earlier studies (Carter et al., 1975; Kennedy et al., 1977; Koppeschaar et al., 1983). However, it is not apparent why injections of T₃ caused these changes in realimentation hyperthyroid lambs during the realimentation period but had no effect on hyperthyroid lambs during the predeprivation, deprivation, or realimentation periods. It is probable that the effects of injecting T₃ may be transient, because N losses during the deprivation period and cumulative N balance were not significantly affected. In addition, the dosage of T₃ required to produce these metabolic effects may vary with the feed intake or energy status of the animal.

Results of these experiments are interpreted to indicate that there is a complex interrelationship among stress, nutrient status, and thyroidal status in ruminants. The effects of T₃ on humoral immunity, N metabolism, and other metabolic variables is dependent not only on the dosage of T₃ used, but also on the nutritional and stress status of the animal.

Implications

During marketing and transport, feeder calves have accelerated tissue losses and decreased immune responses. Products that could reduce these tissue losses and/or improve the immune response could be of benefit to the beef cattle industry. Results of these experiments suggest that, under the conditions of these experiments, injections of triiodothyronine will not have beneficial effects on nutrient losses, nutrient repletion, or the humoral immune response in stressed or morbid calves. Thus, injections of triiodothyronine would not be of benefit to calves subjected to marketing-transport stress.

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