NITROGEN KINETICS OF INFECTIOUS BOVINE RHINOTRACHEITIS- STRESSED CALVES

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ABSTRACT

Eight crossbred feeder steers were used in two consecutive N balance studies to investigate the effects of infectious bovine rhinotracheitis virus (IBRV) on N kinetics. Balance Study 1, which followed a 10-d acclimation phase, consisted of 7 d of sample collection referred to as the healthy phase (d -7 to d -1). Study 2, the IBRV-infected phase, began 2 d after a nasal IBRV challenge and continued for 6 d (d 2 to d 8). A stable isotope, [15N]-glycine, was used to determine N kinetics in both studies. Steers weighed 203 kg at the beginning of the study, 208 kg at IBRV infection and 194 kg at the end of study. Infection with IBRV increased (P < .05) urinary N excretion from 17.9 to 31.5 g/d. Daily N balance was reduced (P < .05) during infection from 21.2 to -3.3 g/d. Total serum proteins increased (P < .05) during infection from 6.6 to 7.1 g/100 ml, the increase being predominantly in the alpha and gamma globulin fractions. Blood urea-N increased (P < .05) during infection from 6.6 to 12.9 mg/100 ml. The urine excretion curve of the stable isotope and the N balance data indicated that IBRV infection increased N turnover and altered tissue utilization of N.

(Key Words: Nitrogen, Metabolism Nitrogen Turnover, Proteins, Infectious Bovine Rhinotracheitis, Stress.)

Introduction

A portion of the feeder cattle marketing system presently involves the assembly of cattle from small farms to auction barns, co-mingling at order-buyer facilities and transportation to feedlots or pastures. During this marketing-transit process, calves are subjected to a variety of environmental stressors, as well as to bacterial and viral pathogens (Hoerlein, 1973). All of these factors combine to increase the susceptibility of calves to bovine respiratory disease (BRD). The infectious bovine rhinotracheitis virus (IBRV) is commonly isolated from calves suffering from BRD outbreaks. When inoculated into healthy susceptible cattle, IBRV has been shown to result consistently in the fever, loss of appetite and depression that are typical of BRD (Cummins and Rosenquist, 1980).

Feeding systems that include increased levels of protein during the first 2 wk following arrival of new cattle at the feedlot decrease medication requirements and death loss while increasing weight gain and performance throughout the feeding period (Cole et al., 1984). Increased knowledge of protein synthesis and catabolism during infection could provide a basis by which to evaluate feeding systems for virally infected cattle that may improve recovery from morbidity.

This research was conducted to investigate the effects of IBRV infection on the N kinetics of feeder calves.

Materials and Methods

Study Protocol. Eight English crossbred steers weighing approximately 200 kg were used in two consecutive N balance studies.
Rectal temperatures, nasal swabs, serum, urine and fecal samples were collected for 7 d following a 10-d acclimation period. These 7 d (d -7 to d -1) were considered the healthy phase of the study. On d 0, each steer was inoculated intranasally with 2.7 x 10^5 plaque forming units (pfu) of virulent IBRV. Rectal temperatures, nasal swabs, serum and fecal samples were collected on d 2 to d 8. These 6 d (d 2 to d 8) were considered the IBRV-infected phase of the study. Urine and fecal samples were collected daily and composited within the healthy phase and the IBRV phase for each steer. Serum was collected post-infection on d 10, 12, 14, 21, 28 and 32.

Calves with BRD have low feed intakes (Hutcheson and Cole, 1986). Morbid calves consume feed at approximately 1% of their body weight; therefore, to reduce variation in N kinetics due to changes in feed intake, daily feed intake was restricted to 1% of body weight during the entire study. Steers were fed their diet (Table 1) in two daily feedings at 0800 and 1500. The steers were maintained in metabolism crates in a thermoneutral environment with ambient temperature maintained at 18°C. When IBRV is administered to cattle, all susceptible cattle in the general area become infected, because IBRV is transmitted easily from animal to animal; thus, infected steers cannot be housed with uninfected steers.

Sample Collection. Blood samples were collected via indwelling jugular vein catheters. Serum and plasma were harvested following centrifugation, and samples that were not immediately analyzed were frozen at -20°C. Urine was passed through two layers of cheesecloth and collected in plastic vessels with 10 ml of 6 M HCl. After measurement of the 12-h urine output, 50 ml were stored at -20°C. The 12-h fecal output was weighed, and 10% of the sample was collected for DM and N analyses.

Infusion. A pulse dose of stable 95% [^{15}\text{N}]glycine was infused at the rate of 2.5 mg/kg BW on d -5 (control) and d 4 (peak fever) of the balance studies to determine N kinetics. Urine [^{15}\text{N}] samples were collected into 5 ml of 6 M HCl as excreted for the first 12 h, hourly for the next 12 h, and every 6 h for the next 24 h postinfusion. Blood samples were collected at 10, 20, 30, 50, 70 and 90
min, 2, 3, 4, 5, 7, 9, 12, 15 and 18 h and 1, 2 and 3 d postinfusion.

Infection. Rectal temperatures were measured daily. Nasal swabs and serum samples were taken on alternate days to determine viral excretion and serum neutralizing antibody formation, respectively.

Sample Analyses. Urinary and fecal N were determined by the micro-Kjeldahl procedure (AOAC, 1970). Nitrogen balance (retention, g) was calculated as follows: \( \text{[g N consumed]} - \text{[g N in feces + g N in urine]} \).

Each urine sample was digested with .2 ml of .8 N H\textsubscript{2}SO\textsubscript{4} until clear in micro-Kjeldahl tubes. Samples were rehydrated with distilled water, and \(^{15}\text{N}\) concentration was determined by mass spectrometry (Halliday and Read, 1981).

Total serum protein (TSP) was determined using a refractometer\textsuperscript{8}. Blood urea-N (BUN) and albumin values were determined using a semi-automated analyzer\textsuperscript{9}. Serum protein fractions were separated by agarose gel electrophoresis\textsuperscript{10} in .05 M barbital buffer at pH 8.6 for 35 min. Proteins were stained with amido black and destained with successive rinses in 5% glacial acetic acid. Six distinct electrophoretic fractions were identified and designated as albumin, alpha 1 and 2, beta 1 and 2 and gamma globulins by their electrophoretic mobility. The relative composition of each fraction was determined as the percentage of optical absorbance at 520 nm using a Corning\textsuperscript{11} densitometer. These percentages were adjusted for hemoconcentration or hemodilution by multiplying the relative composition by the TSP value. In order to use this technique quantitatively, initial samples taken for each animal were used as the control, and subsequent values were compared to initial values (Kirk et al., 1975). All calves were initially (d -7) sero-negative to IBRV using primary bovine embryonic kidney cell monolayer cultures for antibody titer determination according to the procedure of Rosenquist and Loan (1969). The excretion of IBRV in the nasal secretions of each calf was determined by plaque assay (Rosenquist and Loan, 1969).

Statistical Analyses. The experiments was a completely randomized design with time and treatment confounded. Mean differences for serum proteins and peak fever were tested by one-way analysis of variance techniques, comparing each day of the healthy phase to each day of the IBRV phase of the study (Snedecor and Cochran, 1967). The average N balance during the control period (d -7 to d 0) was compared to the average N balance during the infectious period (d 2 to d 8). Nitrogen kinetics were fitted to a two-compartment open model using \(^{15}\text{N}\) excretion in the urine (Notari, 1975). The \(^{15}\text{N}\) urinary excretion curves were calculated from urine values using regressions to fit the equation: \( Y = Ae^{0t} + Be^{-0t}, \) with 0 being equal to the percentage of \(^{15}\text{N}\), A and B constants, 0 being 2.718, and 0 the time of sampling. The rate constant for N catabolism (RC) was calculated as follows: RC = A / (A + B) \( \alpha \) where A = A/(A + B) and B = B/(A + B). The rate constant for urine N excretion (RE) was calculated as follows: RE = \( \alpha /RC \). The rate constant for synthesis (RS) was calculated as follows: RS = \( \alpha + \beta - RC - RE \). The half-life of \(^{15}\text{N}\) glycine was calculated from the equation T\textsubscript{1/2} = \( 0.693/\lambda \), where \( \lambda \) is the decay constant (Chase and Rabinowitz, 1965).

Results and Discussion

The nasal excretion of IBRV, antibody seroconversion and fever (Table 2) all are indicators of an active IBRV infection. Sera from blood taken at the time of virus inoculation were devoid of IBRV antibody, nasal swabs were negative for IBRV and rectal temperatures were normal. A peak mean fever of 40.6°C was observed on d 4 postchallenge. Nasal swabs indicated the IBRV was present at d 2, and serum samples contained antibody to IBRV on d 7 postchallenge.

The determination of the specific electrophoretic serum protein profiles is important in human (Talamo et al., 1982) and small animal clinical biochemistry (Osbaldiston, 1972). The serum electrophoretic pattern of IBRV-infected calves indicated a pattern of lower albumin and beta globulin concentrations and higher alpha and gamma globulin concentrations compared with the uninfected phase (Figure 1). Normal serum electrophoretic profile values within an individual are relatively

\textsuperscript{8}TS meter, American Optical Co., Buffalo, NY.
\textsuperscript{9}Technicon Autoanalyzer II, Tarrytown, NY.
\textsuperscript{10}AC Electrophoresis System, Corning, Corning, NY.
TABLE 2. MEAN BODY WEIGHTS, TEMPERATURES, INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS (IBRV) NASAL EXCRETION AND ANTIBODY TITERS OF IBRV-INFECTED CALVES

<table>
<thead>
<tr>
<th>Sample day</th>
<th>Weight, kg</th>
<th>Rectal temp., °C</th>
<th>Nasal excretion, pfu/ml</th>
<th>Presence of IBRV serum antibody titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7</td>
<td>202</td>
<td>38.8</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>-6</td>
<td>205</td>
<td>38.4</td>
<td>0</td>
<td>-</td>
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<tr>
<td>-4</td>
<td></td>
<td>38.6</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>-2</td>
<td></td>
<td>38.6</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>-1</td>
<td>207</td>
<td>38.5</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>0a</td>
<td>208</td>
<td>38.9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>209</td>
<td>39.0</td>
<td>13,240</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>210</td>
<td>39.1</td>
<td>5,810,230</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>39.7</td>
<td>3,342,613</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>194</td>
<td>38.9</td>
<td>9,412</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>38.7</td>
<td>5,210</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>38.9</td>
<td>5,210</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
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<td>38.7</td>
<td>5,210</td>
<td>+</td>
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<tr>
<td>8</td>
<td></td>
<td>38.9</td>
<td>9,412</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>196</td>
<td>38.7</td>
<td>5,210</td>
<td>+</td>
</tr>
</tbody>
</table>

aIBRV inoculation.

constant over a considerable period of time; thus, a minor change in serum profiles can be a significant indicator of disease (Powanda, 1977). The changes in the serum protein electrophoretic profile may reflect shifts in protein metabolism resulting from the IBRV infection. Nitrogen depletion and repletion studies in rats have demonstrated that the concentration of albumin and alpha globulin fractions decreased and the gamma globulin fraction increased when low or N-free diets were fed. However, beta globulins did not decrease until starvation occurred (Weimer, 1961). Thus, the lower concentration of

Figure 1. Protein fractions (g/100 ml) of steers prechallenge (d -8 to d -1) and postchallenge (d 1 to d 10) with infectious bovine rhinotracheitis virus (IBRV) (d 0).
albumin and beta globulin with the higher concentrations of alpha globulin observed for IBRV-infected calves are not related strictly to N intake. Therefore, these changes may be indicative of infection-induced changes in N metabolism. Broad increases in serum gamma globulins are due to increases in plasma cell production of immunoglobulins (Powanda, 1977). This phenomenon may reflect the severity of the disease process.

The alpha globulin concentrations were highest on d 5 (Figure 1). This increase ($P < .05$) in the alpha fraction may have been due to an increase in the acute-phase proteins, which include alpha 1 anti-trypsin, alpha 1 acid glycoprotein, ceruloplasmin, anti-chymotrypsin, and alpha 2 macroglobulin (Powanda, 1977). Alpha proteins may have a specific involvement in the stress response, as illustrated by ceruloplasmin, a copper-binding and transporting protein (Goldstein et al., 1982). Hrgovic et al. (1975) reported decreases in alpha 2-macroglobulin in calves following transportation, and increases after IBRV infection in the same steers. Alpha 2-macroglobulin binds antigen-antibody complexes and may be responsible partially for the alpha fraction increase observed with IBRV infection in this study. The decrease ($P < .05$) in the beta fraction was due to a decline in the beta 1 proteins; beta 2 proteins remained relatively constant throughout the study. Decreases in the beta 1 fraction in disease states in humans are due to decreases in transferrin levels (Abd-El-Fattah et al., 1981).

Gamma globulin concentrations increased ($P < .05$) at d 5 through d 10 as a result of the IBRV infection (Figure 1). This increase may indicate an increase in antibody production. A significant increase in the gamma globulin fraction was reported by Hrgovic et al. (1975) following transport of calves weighing 300 kg. The immune system has been considered to be a high-priority system during stress, as demonstrated by preferential synthesis of immunoglobulins at the expense of other circulating proteins such as fibrinogen (Stein, 1982).

Serum protein values (6.5 g/100 ml) during the healthy phase were within normal ranges (Ruppanner et al., 1978). However, there was an increase ($P < .05$) in TSP after IBRV inoculation, with peak levels of 7.2 g/100 ml occurring on d 5 (Figure 2). These levels returned to normal by d 10. Increases in TSP observed in calves stressed by shipping (Orr et al., 1984) may be related to the lack of voluntary feed intake observed in morbid calves. However, in our study, feed consumption was not significantly affected by the IBRV infection because all calves were fed at maintenance energy levels. The average daily intake was 2,605 g of DM for the 7 d prior to IBRV inoculation and 2,520 g of DM after inoculation. An increase in TSP from 6.1 to 6.9 g/100 ml was reported by Hrgovic et al. (1975) after an IBRV infection. Our results indicate an overall increase in TSP with IBRV infection, specifically due to increases in the alpha and gamma fractions at the expense of albumin and beta 1 fractions (Figure 1). This pattern follows that observed in BRD-stressed calves (Orr et al., 1984).

Blood urea-N values increased ($P < .05$) from 6.5 initially (d -4) to 12.9 mg/100 ml (d 5) as a result of the IBRV infection (Figure 3). Cole and Hutcheson (1981) reported that fasting increased BUN in steers, and Ruppanner et al. (1978) observed increased BUN concentrations in transported calves. Increases in BUN concentrations, when not accompanied by dietary changes, are indicative of accelerated catabolism of body protein, which may be a response to the stress of the IBRV infection (Wallach, 1974).

Daily N retention was reduced ($P < .05$) concomitant with the peak fever of the IBRV infection (Table 3). Nitrogen consumption did not change during the infection. However, fecal and urinary excretions of N were increased ($P < .05$) from 15.2 and 17.9 to 24.2 and
31.5 g/d, respectively, by the IBRV infection. Thus, there was a net loss of N during the infection (−3.3 g/d), compared with a retention of 21.2 g/d prior to infection. Increases in fecal N due to IBRV could be due to decreases in absorption of dietary N and/or increases in metabolic fecal N. The catabolic loss of N, as demonstrated by the negative N balance during the IBRV infection, may have resulted from an increase in protein breakdown, a block in synthesis, or both. However, the N balance technique measures only the difference between the two processes of intake and outflow without revealing the intermediate N kinetics. Thus, N balance provides an incomplete explanation of protein utilization and requirements during a short-term process such as viral infection.

Theories regarding metabolic N pools have been developed using both plasma and urine concentration of ¹⁵N (Waterlow, 1980). In our experiment, use of [¹⁵N]-glycine as the amino acid and the dosage level used were based on limited published data on laboratory animals and humans. Unfortunately, blood enrichment levels of ¹⁵N were insufficient in our trial to determine a blood excretion curve. Golden and Waterlow (1977) summarized eight published reports and found agreement between plasma and urine methods for determination of N kinetics; thus, urine enrichment levels were used for calculations of N kinetics. Four assumptions are necessary for the calculation of N rate constants: 1) there is a single pool of metabolic N into which the isotope is uniformly distributed; 2) there is no recycling of the ¹⁵N from the protein pool; 3) [¹⁵N]-glycine is a valid tracer for protein N; and 4) the equilibrating pool and recycling are not changed. The calculated rate constants that partition the metabolic N flux into synthesis, degradation and excretion were calculated from %¹⁵N in urine (Figure 4). The %¹⁵N/h urine enrichment ranged from .15 to .8%. The excretion rate of N during the IBRV infection was determined to be .0256, compared with .0143 %¹⁵N/h for healthy steers; thus, 80% more N was excreted in the urine during IBRV infection. The synthesis rate of N was reduced by 51% with IBRV infection (.367 and .189 %¹⁵N/h for normal and IBRV-infected steers, respectively). The N catabolism rate was increased by 43% with IBRV infection (.218 and .312 %¹⁵N/h for normal and IBRV infected steers, respectively). The standard errors were .0119, .073 and .040 %¹⁵N/h, respectively, for rates of N excretion.

### TABLE 3. DAILY NITROGEN INTAKE, FECAL AND URINARY NITROGEN EXCRETION AND NITROGEN RETAINED BY STEERS BEFORE AND DURING INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS (IBRV) INFECTION

<table>
<thead>
<tr>
<th>Item</th>
<th>Healthy</th>
<th>IBRV-infected</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N consumed, g</td>
<td>54.3</td>
<td>52.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Fecal N, g</td>
<td>15.1a</td>
<td>24.2b</td>
<td>2.0</td>
</tr>
<tr>
<td>Urinary N, g</td>
<td>17.9a</td>
<td>31.5b</td>
<td>3.9</td>
</tr>
<tr>
<td>Retained N, g</td>
<td>21.2a</td>
<td>−3.3b</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a,bMeans in the same row without a common letter in their superscript differ (P < .05).
synthesis and catabolism. Both decreased synthesis and increased N catabolism contributed to the urinary N excretion rate of IBRV-infected steers. Infection with IBRV decreased synthesis and increased N catabolism.

Protein turnover information during pathological periods is still limited; however, O'Keefe et al. (1974) reported a small decrease in protein synthesis but no alteration in catabolism rate after abdominal surgery. Kein et al. (1978) measured whole-body protein turnover in patients undergoing elective surgery and observed a 15% decrease in synthesis rate and a moderate N loss. Long et al. (1977) reported an increase in both synthesis and breakdown in three patients with sepsis. Garlick et al. (1980) reported that the effect of infection, specifically fever as a result of vaccination, may increase protein turnover. The half-life of $^{15}$N (infused as $[^{15}$N]-glycine) was decreased ($P < .05$) from 433 min to 198 min during the IBRV infection. This twofold decrease in the half-life represents an increased N metabolism as a result of the IBRV infection.

In summary, IBRV significantly affected the serum chemistry and N kinetics of the feeder calves in this trial. Decreases in serum albumin and beta globulins may be due to a redistribution of priorities for protein synthesis, with available amino acids being used for increased production of the gamma fraction antibodies as well as alpha globulins. Increases in BUN and TSP indicate an increased catabolism of body protein in response to the stressors of IBRV infection. Increased urinary and fecal N excretion values obtained with N balance showed a 75% increase in N excretion, with an 82% increase in N excretion as measured by $%^{15}$N. The use of the stable isotope excretion patterns further defined N kinetics, which showed a 49% decrease in synthesis of protein accompanied by a 43% increase in catabolism of body protein stores. Decreases in absorption of dietary N or increases in intestinal cell sloughing, as shown by fecal N increases, and increases in protein catabolism, as indicated by the isotope excretion curves during IBRV infection, appear to reflect a need for a higher quality and/or quantity of dietary protein during stress and infection states. Additional work on optimal protein levels and amino acid composition of diets for stressed cattle is needed.

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