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Effect of Amino Acid Supplementation on Whole-Body Protein Turnover in Holstein Steers[1]

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ABSTRACT: We used the [15N]glycine single-dose urea end-product technique to measure whole-body protein turnover in six Holstein steers (250 ± 18 kg). Steers were implanted with Revalor-S and continuously infused abomasally with water (4 L/d) or amino acids (AA; in 4 L/d water) in a crossover experiment (two 14-d periods). The AA infusion contained the following (g/d): lysine (5.3), methionine (3.3), threonine (3.2), tryptophan (1.0), histidine (2.1), and arginine (5.5). Steers were fed a diet containing 85% rolled corn, 10% prairie hay, and 1.1% urea (DM basis) at 2.16% of body weight. Nitrogen retention tended (P = .15) to increase with AA infusion, from 27.9 to 32.9 g N/d. Amino acid infusion numerically increased whole-body protein turnover from 168.6 to 183.2 g N/d, protein synthesis from 152.6 to 169.3 g N/d, and protein degradation from 124.7 to 136.4 g N/d. Enhanced protein accretion may have resulted from a larger increase in protein synthesis than in degradation. The tendency for increased N retention is interpreted to suggest that the implanted, lightweight Holstein steers fed a corn-urea diet in our study were able to respond to AA supplementation, suggesting that at least one of the infused AA was limiting in the basal diet. Protein turnover data suggest that cattle, like other animals, may increase protein synthesis and protein degradation in response to supplementation with limiting AA. The [15N]glycine single-dose urea end-product technique for measuring whole-body protein turnover in cattle may be useful.

Key Words: Steers, Nitrogen Balance, Protein Turnover, Amino Acids, Isotopes

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Introduction

Simple techniques for measuring protein turnover in livestock would be useful in defining the effects of metabolic modifiers on protein accretion. Modes of action of individual growth promotants (e.g., steroidal implants, bovine somatotropin, and β-agonists) must be characterized before they can be combined effectively. Combining compounds of diverse actions could demonstrate additive responses.

Techniques tracing the dynamics of protein metabolism use amino acids (**AA**) labeled with either radioactive (Waterlow and Stephen, 1967) or stable isotopes (Picou and Taylor-Roberts, 1969). Direct techniques require tissue samples from the animals to measure deposition of labeled AA. Indirect or end-product techniques, such as the [15N]glycine single-dose urea end-product method, measure label that is excreted. Protein turnover in humans frequently is measured with [15N]glycine because the technique is noninvasive, and the stable isotope is safe (Assimon and Stein, 1992). Using [15N]glycine in livestock would make repeated measures from the same animal possible and would eliminate challenges associated with radioactivity. [15N]Glycine has been used in swine (Salter et al., 1990; Tomas et al., 1992), but it remains to be tested in ruminants.

Our objectives were to 1) evaluate the [15N]glycine single-dose urea end-product technique for measuring whole-body protein turnover in cattle and 2) measure protein turnover and N balance in steers abomasally infused with potentially limiting AA.

Materials and Methods

Steers and Diet. Six Holstein steers (BW ± SD = 250 ± 18 kg) fitted with ruminal cannulas (10 cm i.d.) were used. Steers were implanted with 120 mg of trenbolone acetate and 24 mg of estradiol (Revalor-S®, Hoechst-Roussel Agri-Vet Company, Somerville, NJ) 10 d before the start of the experiment. Steers were housed in an environmentally controlled room

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Table 1. Ingredient and nutrient composition of the diet

Item	%, DM basis
Ingredient	
Rolled corn	85.14
Prairie hay	10.40
Urea	1.13
Limestone	1.50
Potassium chloride	.60
Dicalcium phosphate	.50
Trace mineral salt[a]	.50
Sulfur	.10
Vitamin ADE[b]	.10
Rumensin premix[c]	.02
Tylan premix[d]	.01
Composition[e]	
Crude protein	10.58
Calcium	.72
Phosphorus	.42
Potassium	.73
NE_m, Mcal/kg	2.01
NE_g, Mcal/kg	1.37

[a]Composition (%): NaCl (95 to 99), Mn (>.24), Cu (>.032), Zn (>.032), I (>.007), and Co (>.004).
[b]To provide 4,400 IU vitamin A, 2,200 IU vitamin D, and 13.2 IU vitamin E per kg of diet DM.
[c]To provide 30 mg monensin/kg of diet DM.
[d]To provide 11 mg tylosin/kg of diet DM.
[e]Feedstuffs were analyzed for crude protein; all other compositional values were calculated.

with constant temperature (21°C) and lighting in metabolism crates to facilitate total collection of feces and urine. During the month preceding the experiment, steers were gradually stepped up to a high concentrate diet. The experimental diet (Table 1) contained (DM basis) 10.6% CP, .7% Ca, .4% P, .7% K, 30 mg/kg monensin (Rumensin® 80; Elanco Animal Health, Indianapolis, IN), and 11 mg/kg tylosin (Tylan® 40; Elanco Animal Health). Steers were fed daily in two equal meals (0600 and 1800) with individual DM intakes set at the amounts that steers were consistently consuming up to the start of the experiment when they were given ad libitum access to feed. Orts, if any, were collected each day before the morning feeding, weighed, and stored in a freezer. Samples of individual feed ingredients also were collected daily to determine N and DM intakes. Water was freely available to the steers.

Experimental Treatments. Steers were assigned to two treatments: continuous abomasal infusion of either a mixture of six AA in 4 L of water/d (AA treatment) or 4 L of water/d without the AA (control treatment). The AA treatment contained the following (g/d): L-methionine (3.3), L-lysine-HCl (5.3 g/d lysine), L-threonine (3.2), L-tryptophan (1.0), L-histidine-HCl-H_2O (2.1 g/d histidine), and L-arginine (5.5). Treatments were infused abomasally by peristaltic pump with polyvinyl chloride tubing (.24 cm i.d.) inserted through the ruminal cannula and into the abomasum via the reticulo-omasal orifice. Tubing was held in place in the abomasum with a small rubber flange (9 cm diameter). Infused AA levels were calculated, using the Cornell Net Carbohydrate and Protein model (O'Connor et al., 1993), as the amounts needed by a 250-kg steer to increase gain by an additional 250 g/d. The amount of methionine infused was double the calculated requirement to provide for cysteine synthesis. Also, twice the calculated amount of tryptophan was infused because of the large variation in estimates of tryptophan requirement. Amino acid infusate was prepared as needed by dissolving AA in water and kept refrigerated until use.

Experimental Design. The study was designed as a crossover experiment with two 14-d periods. The first 4 d of each period were allowed for steer adaptation to infusions. Nitrogen status of ruminants adapts rapidly to postruminally infused treatments, making a short adaptation period appropriate (Hovell et al., 1983). Two hours after the morning feeding on d 5, each of three randomly chosen steers was given abomasally a single pulse dose of .65 g of [^{15}N]glycine (98 atom percent excess; Sigma-Aldrich Chemical, St. Louis, MO) in 100 mL of water for measurement of whole-body protein turnover. Concurrently, the remaining three steers were given .31 g of 1,3-[$^{15}N_2$]urea (98 atom percent excess; Sigma-Aldrich Chemical) dissolved in 50 mL of physiological saline and sterilized by Millipore filtration (.45 μm; Millipore, Bedford, MA), administered as a pulse dose into the jugular vein. The [^{15}N]glycine dose contained 125.6 mg of ^{15}N, and the [^{15}N]urea dose contained 148.3 mg of ^{15}N. Prior to isotope infusions, a urine sample was collected from each steer for a 12-h collection period to determine background enrichment of ^{15}N in urinary urea. Then, after isotopes had been administered, urine collection was started afresh into buckets containing approximately 100 mL of 6 *N* HCl to prevent NH_3-N loss. Urine weight was recorded, and representative aliquots (1 to 2% by weight) were collected for the next 4 d, at 12-h intervals for the first 3 d and after 24 h for the last day. Urine samples were kept separate for each collection time, so that ^{15}N excretion in urinary urea could be measured over time. Feces were collected and weighed every 24 h, with 5 to 10% samples being saved and composited for each steer within a period. On the morning of d 10, a 12-h urine sample was collected again from each steer to determine background enrichment of ^{15}N in urinary urea. Steers then were given the ^{15}N source (i.e., glycine or urea) that they had not received initially. This was followed by another 4 d of fecal and urine collections as described previously. Following the final urine collection on d 14, a blood sample was collected (approximately 5 h after the morning feeding) from each steer by jugular venipuncture into heparinized vacuum tubes. Blood samples were processed immediately, and prepared plasma samples were stored frozen for later analysis of AA. Then treatments were switched between steers (control or AA treatment) for

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the start of the second period, which followed the same schedule as the first. All orts, urine, and fecal samples were stored in a freezer (−20°C) pending later analysis.

Calculation of Protein Turnover. Whole-body protein turnover, and consequently protein synthesis and degradation, were measured using the [^{15}N]glycine single-dose urea end-product method (Assimon and Stein, 1992). This procedure is based on the assumptions that a labeled AA will be partitioned mainly between protein synthesis and oxidation in the same proportions as total AA turnover, and that oxidized end products will be excreted quantitatively in the urine. Thus, by measuring the amount of label not incorporated into body tissues, protein turnover (g N/d) can be calculated as rate of N end product excretion in the urine divided by the fraction of the total ^{15}N dose recovered in that end product (Waterlow et al., 1978a). Plateau cumulative recovery of ^{15}N is determined in an end product, such as urea (Fern et al., 1981), after excreted urine is collected long enough to ensure nearly complete clearance of isotope from the body pool of that end product. Thus, total protein turnover was calculated as:

$$Q = d/G$$

where Q is protein turnover (g N/d), d is the rate of urea N excretion (g/d) in urine (i.e., a nitrogenous end product produced from N in the AA pool that is potentially available for protein synthesis), and G is the fractional recovery (%) in urinary urea of ^{15}N from [^{15}N]glycine (i.e., the amount of ^{15}N excreted as a percentage of the total ^{15}N dose given) at the plateau of the cumulative ^{15}N excretion curve over time. The ^{15}N enrichment of every collected urine sample, following tracer dosing, was corrected by subtracting background ^{15}N enrichment.

Whole-body protein turnover was used to calculate whole-body protein synthesis (PS) and degradation (PD) using the relationships: Q = PD + N absorbed = PS + N excretion in urine, and PS − PD = N retention. Thus, PS (g N/d) was calculated as PS = Q − N excretion, and PD (g N/d) as PD = PS − N retention.

We also derived estimates of protein turnover using alternative calculations (Table 4). To determine the extent to which N recycling would affect the fractional recovery of ^{15}N, we infused steers with [^{15}N]urea and measured cumulative excretion of ^{15}N in the urine over time. Instead of using the rate of N excreted as urinary urea (d), we used total urinary N excretion rate to calculate protein turnover and fractional recovery of isotope from [^{15}N]urea to correct for incomplete urea excretion in the urine. Corrected fractional recovery of ^{15}N from [^{15}N]glycine was calculated as:

$$G\text{-corrected} = G/U$$

where G is the fractional recovery (%) in urinary urea of ^{15}N from [^{15}N]glycine, and U is the fractional recovery (%) of ^{15}N from [^{15}N]urea (percentage of total dose given) at the same time point at which urinary recovery of ^{15}N from [^{15}N]glycine had reached a plateau. The G-corrected value accounts for [^{15}N]glycine that was partitioned toward excretion (urea) but did not appear in the urine. The second approach, aimed at minimizing the influence of label recycling, was to linearly regress cumulative ^{15}N (from [^{15}N]glycine) excretion against time for the interval when ^{15}N excretion had reached a plateau. The Y-intercept (time of dosing) of this regression was combined with urea N excretion, whereas the corrected (as before for urea recycling) Y-intercept was combined with total N excretion to calculate protein turnover.

Laboratory Analyses. Orts were dried in a forced-air oven at 55°C for 48 h. Orts and feed samples were ground to pass through a 1-mm screen using a Cyclotec Sample Mill (model 1093, Tecator Inc., Herndon, VA). Orts, feed, urine, and wet fecal samples were analyzed for N by Kjeldahl analysis (AOAC, 1984). Dry matter contents of orts and feed samples were determined by drying samples at 100°C for 24 h. Blood samples were cooled in ice water immediately after collection and then centrifuged at 4°C for 20 min at 5,000 g. Equal amounts of plasma and 10% (wt/vol) sulfosalicylic acid containing norleucine (1 mM) as an internal standard were mixed and cooled on ice for 30 min. Subsequently, the samples were centrifuged at 31,000 × g for 20 min, and the supernatant was frozen at −20°C pending later analysis of AA. Plasma AA were separated by cation exchange HPLC and were measured via fluorimetry following postcolumn *o*-phthalaldehyde derivitization (Beckman System Gold, Beckman, Palo Alto, CA).

To measure ^{15}N enrichment of urinary urea, a .5-mL urine sample was mixed with .5 mL of .14 M K_2HPO_4 to neutralize acid used during urine collection. One milliliter suspension containing 250 mg of preconditioned Dowex 50W-X8 ion exchange resin (100 to 200 mesh, H^+ form; Sigma Chemical, St. Louis, MO) was added to the neutralized urine sample for 15 min to remove free NH_3 by adsorption (Read et al., 1982). A .5-mL sample of the supernatant was transferred to the middle ring of a Conway plate containing 2 mL of .1 N H_2SO_4 in the center well (reagent volumes were chosen for urine samples containing .3 to .6% N). Then, .2 mL of urease type VI (25 units/.2 mL of buffer, pH = 7.25; Sigma Chemical) was added to the sample to convert urea N to NH_3. Plates were incubated for 1 h at 39°C. Subsequently, .4 mL of 40% (wt/wt) NaOH was added to the middle ring and mixed with the urine sample to volatilize NH_3; plates were incubated again for 1 h at 39°C. A .25-mL sample of $(NH_4)_2SO_4$ solution from the center well was transferred to a titer plate, dried with a food dehydrator, and submitted for commercial analysis of

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^{15}N enrichment (N-15 Analysis Service, Department of Agronomy, University of Illinois) by automated mass spectrometry (Mulvaney et al., 1990).

Statistical Analysis. Data were analyzed statistically by analysis of variance using the GLM procedure of SAS (1982). The model included effects of steer, period, and treatment. For cumulative recovery of ^{15}N over time, data were analyzed as a split-plot using the mixed procedure of SAS (1982) with steer × period × treatment as the main plot error term.

Results and Discussion

Daily feed intake of steers during the entire 28-d experiment averaged 5.41 ± .08 kg DM/d. Nitrogen balance data are shown in Table 2. Amino acid infusion tended to increase ($P = .15$) N retention by 18% over the control, from 27.9 to 32.9 g N/d. Urinary N excretion, as a percentage of N apparently absorbed, tended to decrease when steers received the AA treatment, suggesting that fewer AA entering the free AA pool were oxidized. Thus, AA supplementation resulted in a more ideal AA balance at the tissue level.

Assuming a N content of 2.88% in live weight gain of Holstein steers weighing approximately 250 kg (Ainslie et al., 1993), retained N values translate into live weight gains of .97 and 1.14 kg/d for the control and AA treatments, respectively. As a rule, N balance measurements overestimate true protein accretion (Asplund, 1979); N balance overestimated N deposition by sheep, determined by comparative slaughter, by 24% (MacRae et al., 1993). However, in our study, predicted energy-allowable gain (1.23 kg/d; NRC, 1984) for steers fed only the basal diet was higher than gains estimated from N balance. This suggests that protein (AA) limited gain. The favorable response of N balance when steers were supplemented postruminally with AA suggests further that a high corn diet containing only urea as supplemental N (at the optimal level of approximately 1%; Milton et al., 1997) may not have supplied sufficient metabolizable protein of adequate AA composition to implanted, growing Holstein steers.

Protein synthesis requires that all needed AA be available simultaneously (Everson et al., 1989). Thus, the inadequate supply of a single AA may limit protein synthesis. Lysine (Titgemeyer et al., 1988) and methionine (Fenderson and Bergen, 1975) have been regarded as the first-limiting AA in grain-based diets. However, performance studies frequently have shown little or no response when cattle were supplemented postruminally with these AA, leading numerous authors to conclude that lysine and methionine were not limiting in typical feedlot diets (Wright and Loerch, 1988; Healy et al., 1995; Hussein and Berger, 1995). In contrast, responses generally were better to supplementation with a mixture of essential AA (Titgemeyer and Merchen, 1990b) or whole proteins (Houseknecht et al., 1992) as opposed to supplementation with one or two amino acids. Often, lysine and(or) methionine probably are limiting, but only slightly more so than a number of other essential AA, implying that several may be colimiting (Merchen and Titgemeyer, 1992). This could explain the response we observed to supplementing steers with six potentially limiting AA.

Table 2. Nitrogen balance of Holstein steers abomasally infused with a mixture of six amino acids

Nitrogen	Control	Amino acids	SEM	P =
	g of N/d			
Feed	90.1	89.9	1.4	.93
Infused	—	4.2		
Fecal	31.6	30.9	.3	.18
Urinary	30.5	30.3	1.2	.89
Retained	27.9	32.9	2.0	.15

Most ^{15}N excretion in urinary urea occurred within 48 h for both ^{15}N sources. Urinary N excreted as urea averaged 16.0 and 13.9 ± 1.3 g/d for the control and AA treatments, respectively. Because of inappropriate allocations of infusates to steers during the [^{15}N]urea infusion phase and unrealistic data from one steer, excretion data from four steers were not available for calculating the average cumulative recovery of ^{15}N originating from [^{15}N]urea (the average value contains five observations from the control and three from the AA treatment). Steers excreted an average of 60.3 ± 4.7% of the ^{15}N dose given intravenously as [^{15}N]urea within 48 h (Figure 1). Infusing steers abomasally with the AA treatment tended to decrease ($P = .19$) fractional excretion at 48 h of the ^{15}N dose given as [^{15}N]glycine from 9.7 to 7.8 ± .8% (Figure 2). After 48 h, a small amount of ^{15}N from [^{15}N]glycine was excreted steadily over time. We used the 48-h cumulative recoveries to calculate protein turnover.

Table 3 shows that whole-body protein turnover in steers increased numerically from 168.6 (control) to 183.2 g N/d when AA were infused. Generally, protein turnover increases with supplementation of protein (AA) when basal supplies are below an animal's requirement. Increases in protein supply above the requirement affect protein turnover minimally (Salter et al., 1990). As would be expected from the positive N balance response, AA supplementation numerically increased protein synthesis (11%). However, protein degradation also increased numerically (9%). This agrees with reports on other animals, such as pigs, that AA supplementation of protein-deficient animals increases both protein synthesis and protein degradation, but the net result is an increase in protein accretion (Salter et al., 1990). If protein synthesis and degradation rates increased, the tendency for higher protein accretion in steers supplemented with

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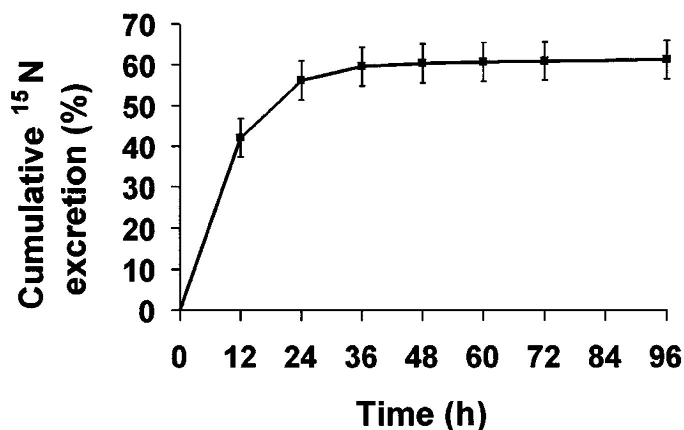

Figure 1. Average (control plus amino acids) cumulative excretion of ^{15}N (percentage of dose given) in urinary urea after steers were dosed with [^{15}N]urea (error bars = SEM).

AA may have resulted from an increase in the difference between these two rates. Efficiency of protein synthesis (i.e., the percentage of synthesized protein that was retained) did not change upon AA infusion (19.1 and 21.1% for the control and AA treatments, respectively; Table 3). Incremental efficiency of protein synthesis (i.e., the change in retained protein expressed as a percentage of the change in protein synthesis with AA infusion) averaged 30%, which agrees well with the values reported by Lobley et al. (1987) for steers and Harris et al. (1992) for lambs as feed intake was increased above maintenance. Whole-body protein synthesis seems to be closely correlated to metabolic size. Buttery (1980) reported a range of 12.5 to 18.9 g of protein synthesized per kilogram of metabolic body weight daily for a wide range of species. In comparison, in our study protein synthesis averaged 15.2 and 16.8 g per kilogram of metabolic body weight daily for the control and AA treatments, respectively.

We compared our estimates for whole-body protein turnover with those reported by Eisemann et al. (1989) because of the similarity in N retention values. Although such a comparison across two independent studies is not a statistical test, it nevertheless serves to show that the [^{15}N]glycine method yielded estimates of protein turnover that are comparable to estimates produced by a more established method. Eisemann et al. (1989) measured whole-body protein turnover by using 1-[^{14}C]leucine in steers treated with bovine somatotropin and fed a corn-based diet. A comparison of results obtained with [^{15}N]glycine and 1-[^{14}C]leucine is significant because the two methods rely on essentially independent assumptions (Waterlow et al., 1978b). Estimates of protein turnover derived from ^{14}C-labeled tracers are based on the kinetics of a single AA in plasma, whereas ^{15}N-labeled tracers yield estimates based on the metabolism of all the AA in the body. Excipient-treated steers in the study of Eisemann et al. (1989) retained 28.8 g N/d, which is comparable to that retained by steers without AA infusion in our study (27.9 g N/d). Injections of bovine somatotropin increased N retention to 38.1 g N/d, approximately twice the response we observed with limiting AA infusion (32.9 g N/d). Whole-body protein synthesis increased from 203 to 225 g N/d by bovine somatotropin treatment, and protein degradation also tended to increase (177 vs 189 g N/d for excipient-vs somatotropin-treated steers).

Estimates of protein turnover derived using alternative calculations are presented in Table 4. Regression analysis of cumulative excretion of ^{15}N against time for the interval 48 to 96 h (from the time that the pseudo-plateau started in Figure 2) yielded Y-intercepts of 8.4 and 6.7 ± .7%, and slopes of .027 and .023 ± .003 %/h for the control and AA treatments, respectively. One of the assumptions of the single-dose [^{15}N]glycine end-product method is that recycling of label from protein breakdown is insignificant. However, it is evident from Figure 2 that cumulative

Table 3. Whole-body protein turnover of steers abomasally infused with a mixture of six amino acids

Item	Control	Amino acids	SEM	P =
	g of N/d			
Protein turnover	168.6	183.2	11.4	.42
Protein synthesis	152.6	169.3	11.2	.35
Protein degradation	124.7	136.4	12.1	.53
Efficiency of CP deposition,[a] %	19.1	21.1	2.5	.60

[a]N retention/protein synthesis × 100.

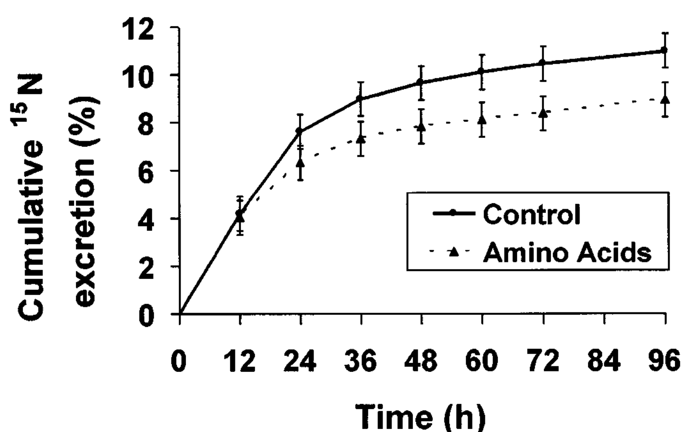

Figure 2. Cumulative excretion of ^{15}N (percentage of dose given) in urinary urea after steers were dosed with [^{15}N]glycine (error bars = SEM).

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Table 4. Estimates of whole-body protein turnover (Q) in steers generated by alternative calculations

Method of calculation[a]					
d[b]	G[c]	Control	Amino acids	SEM	P =
		g of N/d			
Total N, g/d	48 h-corrected	194.9	246.9	19.4	.13
Total N, g/d	Y-intercept$_c$	225.0	294.6	28.4	.16
Urea N, g/d	Y-intercept	194.9	218.8	19.2	.43

[a]Calculated as Q = d/G.
[b]Total N = total urinary N excretion, Urea N = urinary urea N excretion.
[c]48 h-corrected = 48-h recovery of ^{15}N (from [^{15}N]glycine) corrected for urea recycling by dividing by the 48-h recovery of ^{15}N originating from [^{15}N]urea (60.3%); Y-intercept$_c$ = the Y-intercept of the linear regression of ^{15}N recovery against time, for the interval 48 to 96 h after dosing of [^{15}N]glycine, corrected for urea recycling by dividing by the 48-h recovery of ^{15}N originating from [^{15}N]urea (60.3%); Y-intercept = the Y-intercept as calculated above without correction for urea recycling.

excretion of ^{15}N had not reached a true plateau by 96 h. Initially, this small but steady excretion of ^{15}N was probably due to the slow turnover of the body urea pool, but, over time, label recycling from body protein breakdown would contribute increasingly to the amount of ^{15}N excreted. Therefore, subjectively choosing an end point of excretion, such as 48 h, could lead to a small under- or overestimation of protein turnover, because there is no sure way of knowing exactly when label recycling started.

As expected, whole-body protein turnover was estimated to be greater when recovery of ^{15}N from glycine was based on extrapolation of the plateau region (48 to 96 h) to the time of dosing (Y-intercept; Table 4) than when recovery was based on 48-h excretion (Table 3). However, the relationship between treatments was essentially similar with the amino acid infusion leading to numerical increases in protein turnover. When protein turnover was based on total urinary N excretion rather than just urea N excretion (Table 4), flux was higher than when it was based on urea excretion even though excretion of ^{15}N from glycine was corrected for the recovery of ^{15}N from urea (60.3%). This may be due to total N excretion being poorly reflective of the N pool generated from oxidation of amino acids; some N-containing compounds that are excreted in urine result from metabolic processes unrelated to amino acid oxidation (e.g., creatinine, methylhistidine, and hippuric acid). Similar to the flux calculations based on urea N excretion, those based on total urinary N excretion demonstrated tendencies to increase in response to infusion of the amino acid mixture.

The single-dose [^{15}N] glycine technique seems to be a potentially useful method for measuring whole-body protein turnover in cattle. Treatment responses were similar to those expected (though not statistically significant), and values were roughly similar to those of Eisemann et al. (1989) for cattle with similar N retentions. Also, treatment responses showed similar trends regardless of the calculation method that was used. Despite these observations, it is clear that variation among steers was large and that statistical significance between treatments was minimal; this may limit future application of the technique. However, simplification of the procedure by eliminating the [^{15}N]urea infusions and by using a single 48-h collection of urine (as opposed to measuring urinary ^{15}N for each 12-h sample) may reduce some of this variation. The value of the technique will need to be defined better in future studies.

Figure 1 shows that approximately 60% of the urea tracer was excreted in the urine, suggesting that 40% of plasma urea potentially was recycled. This compares well with an estimate of recycled urea N generated by the equation of Kennedy and Milligan (1980) that regressed recycled urea N against dietary crude protein content. Their regression predicts that 31% of urea N in our study would have been recycled to the rumen via saliva and diffusion across the ruminal wall. This value would be higher if urea diffusion to the hindgut was also considered, a factor that our estimate included.

Plasma AA concentrations are presented in Table 5. Plasma AA profiles are influenced by many interacting forces, the net result of which is difficult to predict. Therefore, static measurements of plasma AA levels should be interpreted with caution (Bergen, 1979). Generally, an increase in plasma concentration of any essential AA, upon postruminal protein supplementation, signifies that supply exceeds protein synthetic capacity as dictated by the first-limiting AA. Conversely, a decrease in plasma AA concentrations could imply greater utilization for synthetic purposes (Gibb et al., 1992), because supplementation of a limiting AA should lift previous restrictions that the basal diet may have imposed on protein synthesis. In our study, plasma methionine concentration decreased (P = .06) by 15% in response to the AA treatment. Consequently, methionine was probably not first-limiting in the basal corn-urea diet. Typically, diets containing corn-based proteins supply enough sulfur AA to meet animal requirements (Titgemeyer and Merchen, 1990a). However, the decrease in plasma methionine

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Table 5. Plasma amino acid concentrations of steers abomasally infused with a mixture of six amino acids

Item	Control	Amino acids	SEM	P =
	μM			
Methionine	27.9	23.8	1.1	.06
Lysine	45.5	54.1	1.5	.02
Threonine	78.6	81.6	2.7	.48
Histidine	91.6	91.9	6.3	.98
Arginine	57.2	71.3	4.2	.08
Isoleucine	72.0	56.0	3.4	.03
Leucine	132.6	102.9	4.9	.01
Tyrosine	69.6	57.1	1.3	.003
Phenylalanine	50.7	47.5	1.5	.20
Asparagine	25.6	21.5	.6	.01
Aspartate	31.9	25.4	.9	.01
Serine	80.2	74.7	5.2	.49
Glutamate	299.1	251.3	5.0	.002
Glutamine	35.2	31.8	2.6	.41
Glycine	326.5	333.3	9.8	.65
Alanine	160.5	146.9	5.3	.14
Valine	160.0	132.7	4.7	.02
Citrulline	55.1	53.3	3.2	.70
Ornithine	55.3	49.2	3.4	.27
Total[a]	1,855.0	1,706.1	29.9	.02

[a]Sum of all amino acids listed.

concentration implies that the demand for methionine increased upon AA infusion, suggesting that methionine could have become limiting after AA supplementation (Gibb et al., 1992). Clark (1975) suggested that the essential AA showing the smallest increase in plasma concentration relative to the amount of AA supplemented postruminally could be interpreted as being the most limiting AA. Presumably, then, either threonine or histidine could have been first-limiting in the basal diet of our study. Plasma concentration of lysine increased (P = .02) by 19%, and although it may have been colimiting in the basal diet, lysine accumulation in the blood suggests that supply exceeded requirement after AA infusion. Plasma concentration of arginine was elevated (P = .08) by 25% compared with the control level when steers received the AA treatment, again suggesting that supply exceeded requirement. Most other plasma AA concentrations decreased after AA infusion: isoleucine (–22%; P = .03), leucine (–22%; P = .01), tyrosine (–18%; P = .003), asparagine (–16%; P = .01), aspartate (–20%; P = .01), glutamate (–16%; P = .002), alanine (–9%; P = .14), valine (–17%; P = .02), and total AA (–8%; P = .02). This could reflect increased AA incorporation by peripheral tissues because infusion tended to stimulate protein accretion.

Implications

Nitrogen retention of lightweight Holstein steers tended to increase upon abomasal infusion of potentially limiting amino acids. This indicates the ability of implanted steers to respond to amino acid supplementation when they are fed a high concentrate diet with urea as the sole source of supplemental nitrogen. In cattle, like other animals, protein synthesis and degradation tended to increase in response to supplementation with limiting amino acids; the tendency for improved protein accretion may have resulted from an increase in the difference between these two rates. The [^{15}N]glycine single-dose urea end-product technique deserves further study as a simple method for measuring whole-body protein turnover in cattle.

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