Plasma Amino Acid Response to Graded Levels of Escape Protein[1]

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ABSTRACT: A trial was conducted to examine the potential of using plasma amino acid responses to graded levels of escape protein to determine limiting amino acids in cattle. Growing calves (n = 120; $\bar{x}$ BW = 220 ± 21 kg) were fed a basal diet of corncob:sorghum silage (61:39) and were individually supplemented with distillers' dried grains (DDG), heat-damaged DDG (H-DDG), feather meal (FTH), or urea. The urea supplement was mixed with DDG and H-DDG to allow 0, 20, 35, 50, 65, or 80% of the supplemental CP to come from distillers' protein and maintain an 11.5% CP diet. Urea supplement was mixed with FTH to allow 0, 22, 39, 56, 73, or 90% of the supplemental CP to come from FTH. Dietary CP ranged from 11.5% at the 0% level to 17.3% at the 90% level. Plasma concentration of most essential plasma amino acids responded (P < .10) linearly and/(or) quadratically to increased escape protein. The broken-line response of plasma methionine at low DDG intake suggested that methionine was limiting at low levels of escape protein. An initial decrease followed by a plateau fit by a broken line indicated that histidine became limiting in FTH diets, and lysine eventually became limiting for DDG, H-DDG, and FTH diets before maximum BW gain was reached. Results indicate that plasma amino acid responses may identify amino acids that become limiting with increasing escape protein.

Key Words: Distillers' Grains, Feather Meal, Plasma Amino Acids, Protein Degradation, Beef Cattle

Introduction

The plasma amino acid broken-line technique can be used to identify limiting amino acids of ruminants if ruminal degradation of the supplemented amino acids is avoided (Fenderson and Bergen, 1975; Titgemeyer et al., 1986). However, the need to confine animals in metabolism stalls to enable abomasal infusion of amino acids typically results in few observations in an environment that is quite different from most practical situations. As such, it is difficult to correlate plasma responses with growth responses.

Broderick et al. (1974) demonstrated that lysine, methionine, and valine displayed the broken-line pattern in blood drawn from the mammary vein of dairy cows fed graded levels of ruminally protected casein. The inflection point corresponded with maximum milk production. It is possible that such a pattern would be present for calves fed graded levels of escape protein after maximum gain was reached. If such a pattern were present, it might be possible to determine limiting amino acids as well as requirements in a production environment using this technique.

The following trial was conducted to determine whether the plasma amino acid broken-line technique could be used with calves fed graded levels of escape protein to determine limiting amino acids in a production situation.

Materials and Methods

In a 56-d growth trial, 120 Angus calves ($\bar{x}$ BW 220 ± 21 kg) were individually fed a diet containing forage from sorghum silage-corncob (39:61 ratio) and one of four supplements making up 25.8% of the diet DM (Table 1). Diets were formulated to meet NRC (1984) requirements for CP, energy, vitamins, and minerals for .7 kg/d of gain. Distillers' dried grains (DDG; n = 30), DDG

Table 1. Diet formulation[a]

Ingredient	Urea	Distillers' grains[bc]	Feather meal[d]
Sorghum silage	29.18	29.18	29.18
Corncobs	45.00	45.00	45.00
Molasses	6.30	.50	2.88
Starch	14.71	—	6.71
Urea	2.44	.48	.48
Salt	.30	.30	.30
Dicalcium phosphate	.09	.67	—
Limestone	1.9	.45	.60
Vitamins[e]	.01	.01	.01
Trace minerals[f]	.05	.05	.05
Selenium[g]	.02	.02	.02
Distillers' grains	—	23.34	—
Feather meal	—	—	14.77

[a]Expressed as percentage of DM.

[b]Total diet contained 11.5% CP; the supplement contained 29.1% CP.

[c]Same formulation for heat-damaged distillers' grains. Mixed with urea supplement (25, 44, 63, 81, 100% distillers' grains supplement) to allow 0, 20, 35, 50, 65, or 80% of supplemental CP to come from distillers' grains.

[d]Supplement contained 54.2% CP. Mixed with the urea supplement (25, 44, 63, 81, 100% feather meal supplement) to allow 22, 39, 56, 73, or 90% of supplemental CP to come from feather meal. Dietary CP ranged from 11.5% at 0% level to 17.3% at the 90% level.

[e]15,000 IU of vitamin A, 3,000 IU of vitamin D, and 3.75 IU of vitamin E per gram of premix.

[f]10% Mg, 6% Zn, 4.5% Fe, 2% Mn, .5% Cu, .3% I, .05% Co.

[g]15,000 IU of vitamin A, 3,000 IU of vitamin D, and 3.75 IU of vitamin E per gram of premix.

that had been heat-damaged (24% ADIN) from a malfunctioning dryer (H-DDG; n = 30), or feather meal (FTH; n = 30) were fed at graded levels to obtain growth and plasma amino acid response curves for the different proteins fed. The FTH supplement was 54.2% CP, compared with 29.1% CP for DDG and H-DDG supplements (DM basis). Feather meal protein was fed at higher levels to determine whether increased metabolizable protein is beneficial in obtaining a plasma amino acid broken-line response. Supplements with distillers' grains were mixed with the urea supplement as needed to allow 20, 35, 50, 65, or 80% of the supplemental CP to be supplied by the test proteins while maintaining an 11.5% CP diet. Feather meal supplement was mixed with urea supplement to allow 22, 39, 56, 73, or 90% of the supplemental CP to come from FTH. Total dietary CP increased from 11.5% when no FTH was included to 17.3% at the highest level of FTH inclusion.

A urea supplement was also fed to 30 calves to be used as the zero level of each test protein. Starch and molasses were used as carriers in the supplements to avoid feeding any escape protein other than the test proteins and that in the basal diet.

Calves were fed once daily. All calves received the same amount as a percentage of BW. This amount was adjusted as needed to minimize orts while maintaining intake near ad libitum. Average intake was 2.25% of BW. All calves were implanted with estradiol (Compudose, Elanco, Indianapolis, IN) before the trial. Initial and final weights were the average of three consecutive daily weights. Calves were approximately 12 mo of age and had been restricted in energy intake to gain .2 kg/d for 3 mo before the experiment began.

Blood was drawn by jugular venipuncture from all calves before the morning feeding on d 28, 42, and 56. Blood was placed on ice until it was centrifuged at 5,000 × g for 15 min. Three milliliters of plasma from each calf was deproteinized with 90 mg of sulfosalicylic acid for plasma amino acid analysis. One milliliter of plasma was deproteinized with .125 mL of a 25.6% trichloroacetic acid solution for tryptophan analysis. Plasma samples from each calf were composited and analyzed for amino acid content. Plasma was analyzed for tryptophan fluorimetrically as outlined by Lewis et al. (1976). The remainder of the amino acids were analyzed using ion-exchange chromatography using HPLC equipment (Water, Milford, MA). The same amino acid analysis was used for protein supplements after hydrolysis in 6 N HCl. Protein degradability was determined according to procedures described by Goedeken et al. (1990).

The slope-ratio technique (Klopfenstein et al., 1985) was used to analyze efficiency of protein utilization, and slopes were calculated as units of gain obtained above the urea-supplemented calves for each unit of test protein consumed. Slopes (protein efficiencies) were compared for statistical differences using a two-tailed t-test (Steel and Torrie, 1980). The GLM procedure of SAS (1982) was used to detect linear or quadratic responses of plasma amino acids to increasing levels of protein intake. The quadratic term was removed from the model for those amino acids with a nonsignificant ($P > .10$) quadratic response. Least significant differences (SAS, 1982) were used to separate mean concentrations of amino acids at each level of protein supplementation. Individual plasma amino acid concentrations were plotted against protein intake for each test protein. The NLIN® procedure of SAS (1982) was used to find the breakpoint in plasma concentration of essential amino acids with no initial response to protein intake followed by a positive slope. Such a response is characteristic of a limiting amino acid. Essential amino acids with an initial negative response followed by a plateau were also analyzed for a breakpoint. Such a pattern may identify amino acids that become limiting.

The supply of metabolizable amino acids was estimated at plasma breakpoints. Microbial contribution was estimated using the Burroughs equa-

tion (Burroughs et al., 1974) with an amino acid profile as determined by Goedeken et al. (1990). Metabolizable amino acids from dietary ingredients were estimated based on amino acid profiles, escape values, and N digestibilities. Calculations of amino acids supplied were based on a daily DMI of 2.25% of BW for calves weighing 240 kg (average midtrial BW).

Results

Distillers' dried grains were utilized more efficiently ($P < .01$) than H-DDG or FTH (Figure 1). Maximum gain was reached at .91 kg/d, or .53 kg/d above that of the urea-supplemented calves. Experimental average daily gain was .6 kg/d.

There was a linear increase ($P < .001$) in plasma concentrations of methionine with increased intake of DDG (Table 2). Despite the lack of quadratic response ($P > .65$), convergence on a breakpoint was obtained (Figure 2) at .08 ± .04 kg of DDG Protein. Plasma methionine response to supplemental DDG is characteristic of a limiting amino acid. This breakpoint should indicate the quantity of DDG supplied at the point at which methionine is no longer limiting. The quantity of metabolizable methionine supplied from .08 kg of DDG is only .3 g/d, indicating that methionine was only marginally limiting. The inflection point does not necessarily represent the requirement. As gain increases due to overall response to escape protein, the methionine requirement also increases.
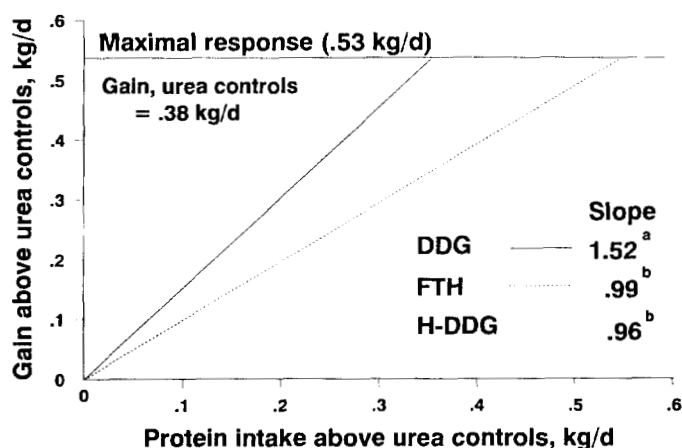

Figure 1. Nonlinear regression of daily intake of test protein against daily gain above urea controls. Resulting values (slopes) are the protein efficiencies. Standard errors of the slopes are .104, .072, and .095 for distillers' dried grains (DDG), heat-damaged distillers' dried grains (H-DDG), and feather meal (FTH), respectively. Values with different superscripts differ ($P < .001$).
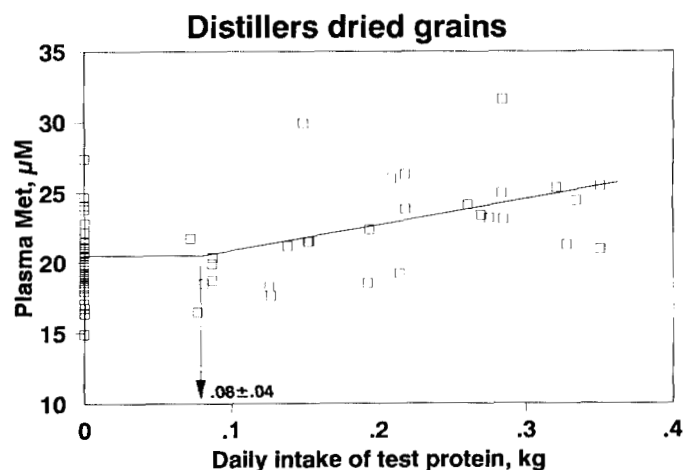

Figure 2. Nonlinear regression of daily intake of distillers' dried grains protein against plasma methionine concentration. The □ represents an animal observation, and the arrow identifies the breakpoint.

There was a linear ($P > .001$) and quadratic ($P < .17$) decrease in plasma concentration of lysine with an increase in supply of DDG. A negative response to supplementation may indicate that increased protein synthesis resulting from supplementation utilized more lysine than was supplied. There was a breakpoint in plasma lysine (Figure 3) at .27 ± .04 kg/d of DDG protein intake, which may indicate that it became limiting at this level of supplementation. Although many trials have demonstrated sulfur amino acids to be limiting for ruminants in diets with low escape protein (Nimrick et al., 1970; Williams and Smith, 1974; Richardson and Hatfield, 1978; Papas et al., 1984; Titgemeyer and Merchen, 1990), lysine is often found limiting in diets high in corn protein (Burris et al., 1976; Hill et al., 1980; Titgemeyer et al., 1988). The DDG used in this trial were from corn. Although the breakpoints do not coincide between methionine (.08 kg of DDG) and lysine (.27 kg DDG), the plasma responses may demonstrate patterns of a first-limiting (methionine with low escape protein) and a subsequently limiting amino acid (lysine with increased corn protein).

A breakpoint in plasma concentration of isoleucine was found for DDG at .07 ± .10. However, the large standard error indicates that this value was not different from zero. Relative to beef tissue, isoleucine is supplied in excess by microbial protein (Ørskov, 1982). Protein synthesis resulting from supplementation with escape protein likely increased utilization of isoleucine and thus decreased excess plasma isoleucine supplied by microbial protein.

Plasma concentration of lysine decreased linearly ($P < .0001$) with increasing H-DDG (Table 3).

Table 2. Plasma amino acid response to supplemental distillers' dried grains[a]

Amino acid	% of Supplemental CP						SEM[c]	Coefficient[b]	
	0	20	35	50	65	80		Linear	Quadratic
Arg	96.0 ± 1.7	91.9	98.8	98.6	88.5	94.6	3.8	—	—
Cys	10.8 ± .5	12.1	14.4	13.5	14.7	12.6	1.1	10.4	−77.9
His	63.5 ± 1.3	58.8	64.8	64.8	63.6	68.3	3.0	—	—
Ile	87.5 ± 1.4	79.1	88.1	79.2	82.3	81.2	3.2	−17.2	—
Leu	100.6 ± 2.3	116.0	157.3	161.6	174.3	183.2	5.2	409.7	−489.0
Lys	99.7 ± 2.4	81.4	77.2	33.5	51.6	54.2	5.5	−148.2	—
Met	20.3 ± .5	19.2	21.7	22.7	25.1	23.5	1.8	13.5	—
TSAA[d]	31.1 ± .8	31.3	36.1	36.2	39.8	36.1	1.9	23.9	—
Phe	44.0 ± .8	46.3	59.5	58.3	64.8	65.2	1.8	70.7	—
Thr	45.1 ± 2.0	41.9	48.7	41.1	42.0	40.8	4.5	—	—
Trp	17.4 ± .7	15.8	18.4	18.5	20.3	20.8	1.6	10.5	—
Val	203.4 ± 3.4	186.1	226.0	195.4	204.2	203.9	7.7	—	—
TEAA[e]	788.4 ± 11.2	748.6	823.3	820.2	831.8	843.3	25.4	184.8	—
TNEAA[f]	1,532.1 ± 32.6	1,399.7	1,560.8	1,429.5	1,411.0	1,329.4	74.0	−447.2	—

[a]Least squares mean concentrations (micromolar).
[b]Only coefficients with $P < .10$ reported. Values represent change in concentration/kilogram of test protein consumed (linear) or (concentration/kilogram)^2 (quadratic).
[c]SEM for 20, 35, 50, 65, and 80% levels based on n = 6 at each level (n = 30 for 0 level).
[d]Total sulfur amino acids (Cys + Met).
[e]Total essential amino acids; includes amino acids listed.
[f]Total nonessential amino acids = Ala, Asn, Asp, Gln, Glu, Gly, Ser, and Tyr.

Convergence on a breakpoint (Figure 4) was established at .29 ± .04 kg/d of H-DDG protein intake despite the low probability for a quadratic response ($P > .35$).

There was a linear decrease ($P < .005$) as well as a quadratic response ($P < .05$) in plasma concentration of isoleucine with increased H-DDG protein intake. A breakpoint was established in plasma isoleucine at low levels of H-DDG supplementation (.08 ± .10 kg/d). As explained for the isoleucine response to DDG, this may not indicate that isoleucine became limiting.

There was a negative linear ($P < .0001$) and quadratic ($P < .0001$; Table 4) response of plasma concentration of histidine to increasing FTH protein. Breakpoint analysis (Figure 5) indicated that histidine became limiting at .15 ± .03 kg/d of FTH protein supplementation. Chalupa et al. (1973) infused several combinations of amino acids abomasally in steers receiving a 60% concentrate diet and found that histidine was required, along with lysine and threonine, to obtain maximum N retention. Storm and Ørskov (1984) suggested that histidine may be one of the first-limiting amino
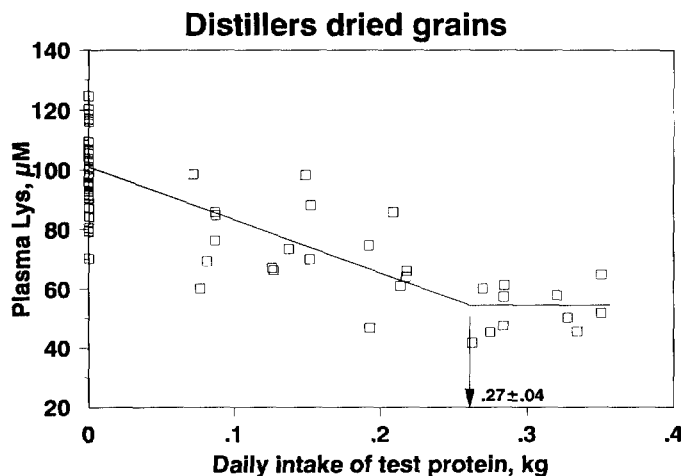

Figure 3. Nonlinear regression of daily intake of distillers' dried grains protein against plasma lysine concentration. The □ represents an animal observation, and the arrow identifies the breakpoint.
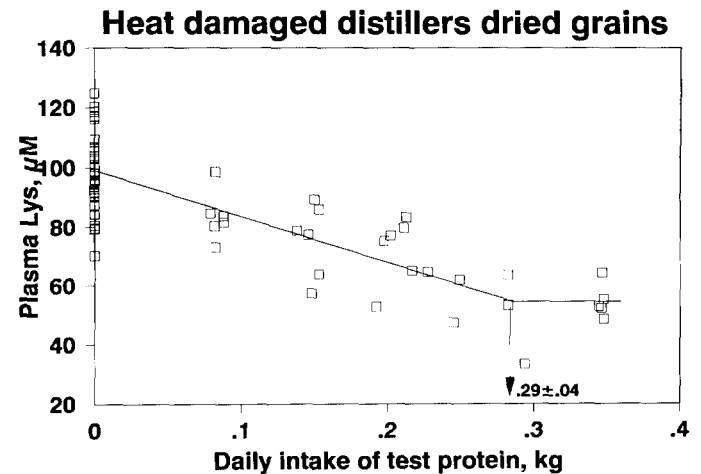

Figure 4. Nonlinear regression of daily intake of heat-damaged distillers' dried grains protein against plasma lysine concentration. The □ represents an animal observation, and the arrow identifies the breakpoint.

Table 3. Plasma amino acid response to supplemental heat-damaged distillers' dried grains[a]

| Amino acid | % of Supplemental CP | | | | | | SEM[c] | Coefficient[b] | |
	0	20	35	50	65	80		Linear	Quadratic
Arg	95.0 ± 1.9	88.6	96.2	98.1	86.4	87.1	4.2	—	—
Cys	10.7 ± .5	13.2	12.0	14.5	14.8	13.8	1.0	12.16	—
His	63.9 ± 1.2	60.1	67.1	63.2	62.4	59.1	2.6	—	—
Ile	86.7 ± 1.3	80.4	79.3	83.7	79.9	81.2	2.9	−73.5	183.92
Leu	100.2 ± 2.6	117.4	136.3	163.6	174.4	180.5	5.7	260.8	—
Lys	98.5 ± 2.3	83.6	75.3	72.1	58.1	51.1	5.1	−142.8	—
Met	20.1 ± .5	19.3	20.3	23.1	24.8	22.9	1.1	11.2	—
TSAA[d]	30.8 ± .9	32.5	32.3	37.6	39.6	36.8	1.9	23.6	—
Phe	43.5 ± .8	49.5	50.0	59.0	64.0	63.8	1.8	64.9	—
Thr	44.6 ± 20.1	44.4	39.4	45.1	35.2	36.0	4.7	−29.2	—
Trp	17.1 ± .9	17.3	18.7	18.9	20.4	17.9	1.9	—	—
Val	202.5 ± 3.2	191.1	189.4	207.2	209.2	202.6	7.1	−95.8	355.4
TEAA[e]	782.6 ± 12.9	764.9	784.1	848.7	829.7	815.9	28.5	153.1	—
TNEAA[f]	1,519.5 ± 32.7	1,422.8	1,440.5	1,469.8	1,451.6	1,266.2	72.0	−558.7	—

[a]Least squares mean concentrations (micromolar).
[b]Only coefficients with $P < .10$ reported. Values represent change in concentration/kilogram of test protein consumed (linear) or (concentration/kilogram)^2 (quadratic).
[c]SEM for 20, 35, 50, 65, and 80% levels based on $n = 6$ at each level ($n = 30$ for 0 level).
[d]Total sulfur amino acids (Cys + Met).
[e]Total essential amino acids; includes amino acids listed.
[f]Total nonessential amino acids = Ala, Asn, Asp, Gln, Glu, Gly, Ser, and Tyr.

acids of microbial protein. Based on the amino acid profile of beef tissue, Owens (1986) suggested that microbial protein may be limiting in histidine, leucine, lysine, and sulfur amino acids. Ørskov (1982) pointed out that microbial protein is low in histidine compared with beef tissue. The response curve obtained does not suggest that histidine is limiting in the control diet, but it does suggest that histidine rapidly becomes limiting with increased

protein synthesis. Even with the high escape value, FTH is a very poor source of metabolizable histidine (Table 5). This may explain why histidine seems to be limiting for FTH but not for the distillers' grains.

Plasma concentrations of lysine decreased linearly ($P < .0001$) and quadratically ($P < .02$) with increasing intake of FTH protein. Plasma concentrations stabilized at .33 ± .05 kg of FTH protein

Table 4. Plasma amino acid response to supplemental feather meal[a]

| Amino acid | % of Supplemental CP | | | | | | SEM[c] | Coefficient[b] | |
	0	22	39	56	73	90		Linear	Quadratic
Arg	95.8 ± 2.0	103.8	101.3	116.0	122.4	115.1	4.5	45.2	—
Cys	10.7 ± .4	11.4	11.4	13.5	16.4	14.4	1.0	8.4	—
His	63.6 ± 1.1	39.9	36.0	31.6	34.4	24.1	2.6	−148.0	154.9
Ile	87.1 ± 2.1	107.0	112.6	138.0	157.0	162.7	4.8	140.4	—
Leu	100.2 ± 2.5	133.6	167.0	199.8	203.1	5.7	5.7	—	—
Lys	98.9 ± 2.4	77.8	64.2	55.3	58.3	42.7	5.4	−173.6	148.5
Met	20.1 ± .5	18.2	17.1	16.3	16.3	14.6	1.0	−9.7	—
TSAA[d]	30.8 ± .2	29.6	28.5	29.8	32.7	29.1	1.7	—	—
Phe	43.8 ± 1.0	48.5	54.3	59.2	69.4	69.9	2.3	48.2	—
Thr	44.7 ± 2.6	50.3	61.4	60.5	80.8	70.4	5.9	56.5	—
Trp	17.1 ± .8	19.9	18.2	20.1	19.2	17.2	1.8	—	—
Val	202.5 ± 6.7	297.6	353.9	467.3	549.8	597.3	15.3	724.7	—
TEAA[e]	784.5 ± 15.7	894.5	892.9	1,144.8	1,323.6	1,331.3	35.8	1,022.53	—
TNEAA[f]	1,521.9 ± 38.8	1,564.9	1,392.7	1,491.2	1,260.8	1,413.2	88.1	−340.1	—

[a]Least squares mean concentrations (micromolar).
[b]Only coefficients with $P < .10$ reported. Values represent change in concentration/kilogram of test protein consumed (linear) or (concentration/kilogram)^2 (quadratic).
[c]SEM for 22, 39, 56, 73, and 90% levels based on $n = 6$ at each level ($n = 30$ for 0 level).
[d]Total sulfur amino acids (Cys + Met).
[e]Total essential amino acids; includes amino acids listed.
[f]Total nonessential amino acids = Ala, Asn, Asp, Gln, Glu, Gly, Ser, and Tyr.

Table 5. Metabolizable amino acids supplied by base diet and highest level of protein supplement (g/d)[a]

Amino acid	Base diet[b]	Distillers' grains	Heat-damaged distillers' grains	Feather meal
His	5.5	4.5	3.5	2.5 (1.4)[c]
Lys	28.7	4.8	3.3	6.7 (3.9)
Met	10.6	3.6	2.8	2.1 (1.2)

[a]Calculated from amino acid content, protein escape values, and N digestibility (Table 6). Assumed as DMI of 5.4 kg/d.

[b]Calculated from amino acid content of sorghum silage and corncobs (Table 6) plus estimated available microbial protein based on the Burroughs equation (Burroughs et al., 1974) with an amino acid content as determined by Goedeken et al. (1990).

[c]Values in parentheses are quantities supplied by an equal quantity of feather meal protein as distillers' grains protein.

(Figure 6). This may be the point at which the histidine requirement is met and lysine becomes limiting. The lack of an increase in plasma histidine at this level may be due to the low levels supplied by FTH. Accumulation in the plasma is likely to be very moderate because body protein synthesis would continue to utilize the majority of the histidine supplied.

Discussion

It seems that for all three proteins, lysine may have been limiting immediately prior to the point of maximum gain. The tendency of responses to be quadratic with supplementation ($P <$ .37, $P <$ .17, and $P <$ .02 for H-DDG, DDG, and FTH, respectively), along with the fact that distillers' grains (from

corn) and FTH are poor sources of lysine, supports this theory.

An increase in plasma lysine at maximum gain may not have been detected due to few observations beyond this point. Maximum gain was not achieved until .35 kg/d of DDG protein was fed. The maximum quantity of DDG and H-DDG fed was .38 kg/d. Maximum gain would not have been reached until .55 kg/d of H-DDG protein was fed. Maximum gain for calves fed FTH was reached at .54 kg/d of FTH protein. Maximum quantity of FTH fed was .59 kg/d.

Based on the supply of lysine at maximum gain, it is possible to estimate the lysine requirement. Based on the amino acid content, escape value, and N digestibilities (Table 6), it can be estimated that DDG, H-DDG, and FTH supplied 4.0, 4.4, and 6.7 g of metabolizable lysine, respectively, when maximum gain was reached. When lysine from the basal diet and microbial protein is added to the calculation, this is 33.3, 32.3, or 35.4 g/d. The fact that similar quantities of lysine were supplied at maximum gain for all three proteins supports the theory that it was limiting. Actual available lysine supplied by the FTH diet at the point of maximum gain may be lower than this because N digestibility used in the calculation may overestimate lysine digestibility in FTH (Knabe et al., 1989). Based on the supply of amino acids at maximum gain for several diets, Klopfenstein et al. (1991) estimated a lysine requirement of 33.9 g/d for 283-kg steers gaining .74 kg/d. Fenderson and Bergen (1975) found that plasma lysine increased linearly in a 274-kg steer gaining .73 kg/d with lysine infusion. Based on the metabolizable lysine supplied, they estimated the lysine requirement to be < 23 g/d. Titgemeyer et al. (1988) determined a lysine re-
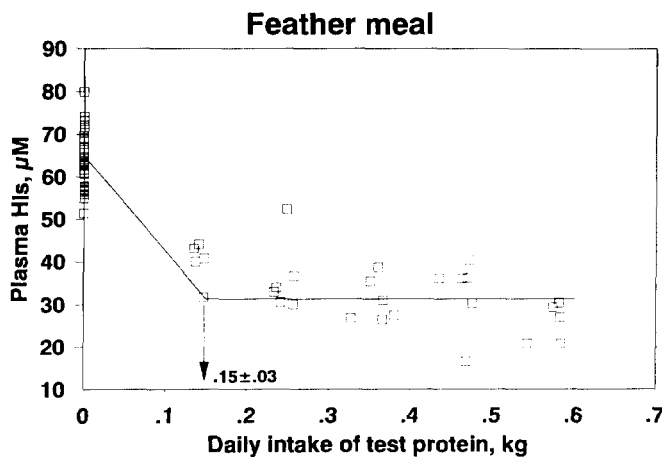

Figure 5. Nonlinear regression of daily intake of feather meal protein against plasma histidine concentration. The □ represents an animal observation, and the arrow identifies the breakpoint.
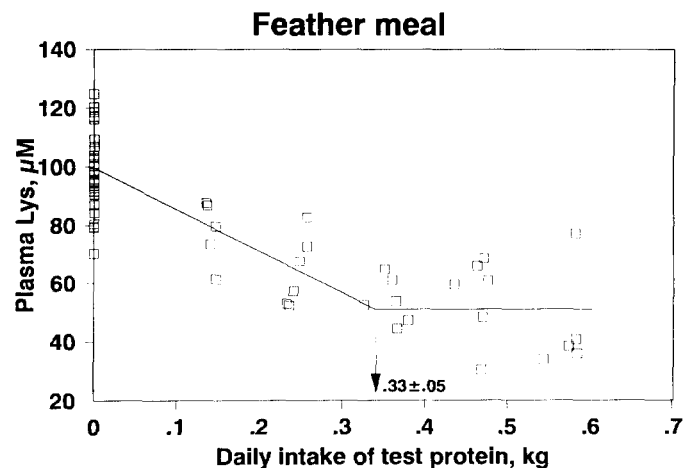

Figure 6. Nonlinear regression of daily intake of feather meal protein against plasma lysine concentration. The □ represents an animal observation, and the arrow identifies the breakpoint.

Table 6. Crude protein, escape protein, N digestibility,
and amino acid content[a] of feeds

Item	Corncobs	Sorghum silage	Heat-damaged distillers' grains	Distillers' grains	Feather meal
CP, %	2.4	7.5	29.0	28.9	84.3
N escape[b]	37.0	17.1	45.8	50.1	66.4
N digestibility, %[c]	—	—	92.0	100.0	94.0
Arg	10.9	6.3	4.0	4.4	6.7
His	1.3	1.7	2.2	2.4	.8
Ile	1.6	3.4	2.0	3.1	3.0
Leu	4.3	7.6	11.4	11.9	7.7
Lys	2.6	4.7	1.9	2.3	2.0
Met	1.5	1.5	1.7	1.9	.6
Phe	2.4	4.1	5.0	5.2	4.9
Thr	3.5	4.2	3.4	3.6	4.1
Trp	ND[d]	.6	.7	.6	.4
Val	3.0	4.1	4.2	4.2	6.2
Cys	1.9	1.9	1.6	1.8	6.2
TSAA[e]	3.4	3.4	3.3	3.7	6.8

[a]Expressed as a percentage of CP.
[b]Percentage of protein remaining after 12 h in situ.
[c]Determined by Nakamura et al. (1991).
[d]Not determined.
[e]Total sulfur amino acids (Met + Cys).

quirement of 44 to 48 g/d for a 383-kg steer gaining 1.08 kg/d.

Identification of methionine as initially limiting for DDG-supplemented calves (Figure 2) is supported by previous research that has identified sulfur amino acids as limiting in low-protein diets (Nimrick et al., 1970; Williams and Smith, 1974; Richardson and Hatfield, 1978; Storm and Ørskov, 1984). Such a broken-line response, however, was not expected for any amino acid until maximum gain was reached.

A broken-line response of a limiting amino acid is not as distinguishable using whole proteins as it is when a single limiting amino acid is supplied. When an infused limiting amino acid meets the requirement, either energy or a subsequent amino acid limits further protein synthesis, allowing rapid accumulation in the plasma of the infused amino acid. When whole proteins are fed and an amino acid requirement is met, the subsequent limiting amino acid continues to be supplied and continued protein synthesis moderates accumulation in the plasma.

It is not feasible to conclude that methionine is limiting in diets low in escape protein based on these data (Tables 2, 3, and 4). A broken-line response should also have been observed for H-DDG and FTH, because the control calves fed urea represented the zero level of each test protein. A linear decrease in plasma methionine for FTH (Table 4) indicates that methionine or total sulfur amino acids are likely not limiting at low levels of escape protein.

Initial negative responses of amino acids to protein intake followed by a plateau (lysine for DDG and H-DDG, histidine and lysine for FTH) may be of more value than responses that identify amino acids that are limiting at low levels of escape protein. The quadratic significance of these responses adds credence to the obtained breakpoints. Such responses may identify amino acids that are limiting in the escape protein per se and not just in the microbial protein. If the biological value of the absorbed microbial protein is as high as has been estimated ("near 100"; Owens and Zinn, 1986), the limiting amino acid in microbial protein may be inconsequential to livestock production. Determining amino acids that become limiting with supplementation with different proteins may be more valuable for productive purposes.

Implications

Plasma amino acid patterns may identify amino acids that become limiting with escape protein supplementation. The response of plasma methionine to graded dietary levels of distillers' dried grains may indicate that methionine is limiting in microbial protein. Histidine may limit protein utilization of feather meal at moderate levels of supplementation. Lysine may ultimately become limiting with increased supplementation of feather meal, distillers' dried grains, and heat-damaged distillers' dried grains.

Literature Cited

Broderick, G. A., L. D. Satter, and A. E. Harper. 1974. Use of plasma amino acid concentration to identify limiting amino acids for milk production. J. Dairy Sci. 57:1015.

Burris, W. R., J. A. Boling, N. W. Bradley, and A. W. Young. 1976. Abomasal lysine infusion in steers fed a urea supplemented diet. J. Anim. Sci. 42:699.

Burroughs, W., A. H. Trenkle, and R. L. Vetter. 1974. A system of protein evaluation for cattle and sheep involving metabolizable protein (amino acids) and urea fermentation potential of feedstuffs. Vet. Med. Small Anim. Clin. 69:713.

Chalupa, W., J. E. Chandler, and R. E. Brown. 1973. Abomasal infusion of mixtures of amino acids to growing cattle. J. Anim. Sci. 37:339 (Abstr.).

Fenderson, C. L., and W. G. Bergen. 1975. An assessment of essential amino acid requirements of growing steers. J. Anim. Sci. 41:1759.

Goedeken, F. K., T. J. Klopfenstein, R. A. Stock, and R. A. Britton. 1990. Hydrolyzed feather meal as a protein source for growing calves. J. Anim. Sci. 68:2945.

Hill, G. M., J. A. Boling, and N. W. Bradley. 1980. Postruminal lysine and methionine infusion in steers fed a urea-supplemented diet adequate in sulfur. J. Dairy Sci. 63:1242.

Klopfenstein, T., R. Stock, and R. Britton. 1985. Relevance of bypass protein to cattle feeding. Prof. Anim. Scientist 1:27.

Klopfenstein, T., V. Wilkerson, D. Gibb, and T. Nakamura. 1991. Beef and amino acids. In: Proc. from the National Feed Ingredients Association Amino Acid Symp. Chicago, IL.

Knabe, D. A., D. C. LaRue, E. J. Gregg, G. M. Martinez, and T. D. Tanksley, Jr. 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci. 67:441.

Lewis, A. J., P. J. Holden, R. C. Ewan, and D. R. Zimmerman. 1976. An automated fluorometric method for the measurement of tryptophan in plasma. J. Agric. Food Chem. 24:1081.

Nakamura, T., T. J. Klopfenstein, D. J. Gibb, and R. A. Britton. 1991. Growth efficiency and digestibility of heat-damaged distillers dried grain in growing cattle. J. Anim. Sci. 69(Suppl. 1):148 (Abstr.).

Nimrick, D., E. E. Hatfield, J. Kaminski, and F. N. Owens. 1970. Quantitative assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. J. Nutr. 100:1295.

NRC. 1984. Nutrient Requirements of Beef Cattle (6th Ed.). National Academy Press, Washington, DC.

Ørskov, E. R. 1982. Protein Nutrition in Ruminants. Academic Press, London.

Owens, F. N. 1986. Protein utilization in ruminants: Current concepts in formulating ruminant diets. In: Proc. Am. Feed Ind. Assoc. Nutr. Symp. p 12. St. Louis, MO.

Owens, F. N., and R. Zinn. 1986. Protein metabolism of ruminants. In: D. C. Church (Ed.) The Ruminant Animal, Digestive Physiology and Nutrition. p 227. Prentice-Hall, Englewood Cliffs, NJ.

Papas, A. M., J. L. Vicini, J. H. Clark, and S. Pierce-Sander. 1984. Effect of rumen-protected methionine on plasma free amino acids and production by dairy cows. J. Nutr. 114:2221.

Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. J. Anim. Sci. 46:740.

SAS. 1982. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.

Steel, R.G.D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Book Co., New York.

Storm, E., and E. R. Ørskov. 1984. The nutritive value of rumen micro-organisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. Br. J. Nutr. 52:613.

Titgemeyer, E. C., and N. R. Merchen. 1990. Sulfur-containing amino acid requirement of rapidly growing steers. J. Anim. Sci. 68:2075.

Titgemeyer, E., N. R. Merchen, and L. L. Berger. 1986. Determining the lysine and methionine requirements of growing steers fed corn grain-based diets. J. Anim. Sci. 63(Suppl. 1):405 (Abstr.).

Titgemeyer, E. C., N. R. Merchen, L. L. Berger, and L. E. Deetz. 1988. Estimation of lysine and methionine requirements of growing steers fed corn silage-based or corn based diets. J. Dairy Sci. 71:421.

Williams, A. P., and R. H. Smith. 1974. Concentrations of amino acids and urea in the plasma of the ruminating calf and estimation of the amino acid requirements. Br. J. Nutr. 32:421.