Dietary protein reduction on microbial protein, amino acid digestibility, and body retention in beef cattle: 2. Amino acid intestinal absorption and their efficiency for whole-body deposition

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ABSTRACT: The objective of this study was to determine the apparent and true intestinal digestibility of total and individual AA, and to estimate the efficiency of whole-body AA retention from individual and total absorbed AA. Four Nellore animals (241.3 kg initial BW) and four crossbred Angus × Nellore (263.4 kg initial BW) cannulated in rumen and ileum were randomly allocated in two 4 × 4 Latin squares. The experiment lasted four 17 d periods, with 10 d for adaptation to diets and another 7 d for data collection. The diets consisted of increasing CP levels: 100, 120, or 140 g/kg of DM offered ad libitum, and restricted intake diet with 120 g CP/kg DM (experiment 1). In experiment 2, forty-four bulls (22 Nellore and 22 crossbred F1 Angus × Nellore) with 8 months and initial shrunk BW 215.0 ± 15.0 kg (Nellore = 208.0 ± 12.78 kg; Angus × Nellore = 221.9 ± 14.16 kg) were used. Eight of those animals were slaughtered at the beginning of the experiment. The remaining 36 bulls were allocated in a completely randomized design with six replicates, in a 2 (genetic groups) × 3 (CP contents) factorial scheme. The amount of essential AA (EAA) and nonessential AA (NEAA) reaching the small intestine increased linearly ($P < 0.05$) in response to CP content. The apparent digestibility of EAA was not affected ($P > 0.05$) by CP content, with exception for histidine ($P = 0.07$, linear effect), leucine ($P = 0.01$, linear effect), and methionine ($P = 0.05$, linear effect). Differences existed among AA when compared the apparent digestibility of NEAA. The apparent digestibility of alanine ($P = 0.05$), aspartic acid ($P = 0.07$), glutamic acid ($P = 0.02$), glycine ($P = 0.05$), proline ($P = 0.02$), and serine ($P = 0.04$) responded quadratically to CP content increase. However, the apparent digestibility of cystine and tyrosine was not affected ($P > 0.05$) by increasing dietary CP. The true intestinal digestibilities of total, essential, nonessential AA, lysine, and methionine were 75.0%, 77.0%, 74.0%, 77.0%, and 86%, respectively. The true intestinal digestibility of total microbial AA was 80%. The efficiency of utilization of total AA for whole-body protein deposition was 40%. The efficiency of utilization of lysine and methionine was 37% and 58%, respectively. It was concluded that the AA flow to the omasum increases in response to dietary CP content. In addition, there are differences among AA in the efficiency that they are used by beef cattle.

Key words: beef cattle, individual AA, lysine, methionine, small intestine

INTRODUCTION

The AA available to the ruminant animal is a product of the amount of AA reaching the small intestine (**SI**) and their digestibility at that site (Tigmeyer, 1990). However, feeding systems are constrained by limited knowledge about the efficiency of absorbed AA utilization and usually assume a unique value for total AA digestibility.

Tas et al. (1981) determined that true digestibility of microbial AA was 86% in sheep, and Tamminga (1980) for lactating and non-lactating cattle found 82% and 83%, respectively. Furthermore, little information is available for individual intestinal digestibility for AA, and there is a need to accurately obtain these values to improve ruminant feeding systems.

Protein requirements in ruminants are based on net protein deposition and its efficiency of utilization; AA requirements are based on net protein requirement and AA profile in whole-body tissue (Titgemeyer and Loest, 2001). However, the efficiency in which absorbed AA are used for protein retention is difficult to access (NASEM, 2016), and there are few and contradictory estimates. According to the AFRC (1993), the efficiency of AA usage for gain was assumed to be 59% for all essential AA (**EAA**), while the Brazilian system (BR-Corte, Valadares Filho et al., 2016) suggested 47.4% as efficiency of MP, which is near to that of 49% mentioned in NASEM (2016). Despite these systems adopted the same values for efficiency for AA usage, recent evaluations showed that differences exist among individual AA (Titgemeyer et al., 1990; Löest et al., 2002; Batista et al., 2016).

We hypothesized that there are differences between efficiencies of each AA utilization for whole-body protein retention, and that intestinal digestibility can vary depending on the AA type. Therefore, the objective of this study was to determine the intestinal digestibility of individual AA. In addition, our objective was to determine the efficiency of individual AA utilization for whole-body retention in beef cattle.

MATERIALS AND METHODS

Experiment 1. Digestion Trial

Animals, experimental design, and diets. All procedures regarding the animals handling and cannulation were approved by the Institutional Animal Care and Use Committee at Federal University of Viçosa (CEUAP/UFV), with protocol number 06/2013. The ileal cannulation procedure was described by Leão and Coelho da Silva (1980). The research was conducted at the Experimental Feedlot of the Animal Science department of the Federal University of Viçosa (UFV), Brazil.

Eight bulls, four Nellore (241.3 ± 43 kg of BW; 13 mo) and four crossbred Angus × Nellore (263.4 ± 47 kg BW; 13 mo), with rumen and ileum cannulas, were distributed in two 4 × 4 Latin squares, each for one genetic group. The experiment was conducted in four 17-d periods, being 10 d for diet adaptation and 7 d for data collection/sampling. Bulls were housed in tie stalls (8 m^2) with free access to feed and water throughout the experiment.

Experimental diets consisted of increasing dietary CP content: 100, 120, or 140 g CP/kg DM offered ad libitum, and another control diet containing 120 g CP/kg DM, offered through restricted intake (**RI**) at 41.5% of restriction compared with ad libitum consumption. The RI treatment was used to evaluate the influence of intake level on microbial efficiency. Also, RI was used to quantify the true intestinal digestibility of AA. Experimental diets were composed of 50% corn silage (**CS**) and 50% concentrate, in DM basis. For animals in voluntary intake, the total daily amount of CS was provided at 0700 h with half of the daily amount of the concentrate. The other half was provided at 1500 h (Pazdiora et al., 2014). Feed intake was adjusted to keep orts within 5% to 10% of the amount offered. The animals in RI were fed once a day at 0700 h. Experimental diets were formulated according to the Brazilian System of Nutrient Requirements of Zebu Beef Cattle—BR-CORTE, described by Valadares Filho et al. (2010), to allow an ADG of 1 kg/d (Tables 1 and 2).

Experimental procedures and sampling preparation. The CS and orts were sampled from the 10th d to 16th d of each experimental period and partially dried in a forced air ventilation oven (55 °C) for 72 h. After drying, the samples were ground in a Wiley mill (TE-648, TECNAL, Piracicaba, Brazil) with 2 and 1 mm mesh and composed proportionally to dry weight, per animal per period. The ingredients that composed the concentrate were sampled directly from the silos, in the days they were mixed. These samples were stored at −20 °C for later chemical analyses.

The double marker method (France and Siddons, 1986) was used to estimate omasal digesta flow. Two markers were used: Co-EDTA, which

Table 1. Chemical composition of the diets and ingredients proportion

	Dietary CP (g/kg DM)		
Item	100	120	140
Ingredient, g/kg DM			
CS	500	500	500
Corn grain	397	396	396
Wheat meal	60	30	0
Soybean meal / U+AS[1]	22	53	83
Sodium chloride	5	5	5
Mineral mixture[2]	5	5	5
Sodium bicarbonate	8	8	8
Magnesium oxide	3	3	3
Chemical composition, g/kg DM			
OM	954	949	944
CP	99	121	142
Ether extract	40	40	40
$_{ap}$NDF	314	307	299
NFC	507	494	482
RDP, g/kg CP[3]	668	696	716

[1]U + AS = urea + ammonia sulfate; 83.3% of soybean meal and 16.7% of urea + ammonia sulfate.

[2]Mineral mixture = 223 g/kg calcium; 174 g/kg phosphorus; 24 g/kg sulfur; 100 mg/kg cobalt; 1,250.0 mg/kg copper; 1,795.0 mg/kg iron; 90 mg/kg iodine; 2,000.0 mg/kg manganese; 15.00 mg/kg selenium; 5,270.00 mg/kg zinc and 1,740.00 mg/kg fluor.

[3]RDP = calculated based on the Brazilian Feed Composition Tables for Ruminants (Valadares Filho et al., 2017), considering Kp = 0.05.

is associated with the liquid phase and small particle phase, and iNDF, which is associated with the large particle phase. The iNDF was used as a single marker to estimate the ileal DM flow. Continuous infusions of Co-EDTA were performed from the 11th d of each experimental period until the last digesta sampling on 16th d. The total amount of 5.0 g/d of Co-EDTA, or 0.7 g Co, was diluted in 2.7 liters of water and infused through ruminal cannulas by using two peristaltic pumps (BP-600.4; MILAN SCIENTIFIC EQUIPMENT, Inc., Colombo, Paraná, Brazil).

Also, a total of 7.03 g of ammonium sulfate enriched with 10% of ^{15}N atoms [ammonium sulfate ($^{15}NH_4)_2SO_4$] [Sigma Aldrich (Isotec), Miamisburg, OH] was added to the previously described Co-EDTA solution, providing a daily supply of 150 mg of ^{15}N to each animal. In all periods, the ^{15}N ammonium sulfate infusion began 60 h prior to the first digesta sampling to allow uniform distribution of ^{15}N into the $^{15}NH_3$ in the ruminal microbial pool (Broderick and Merchen, 1992). Infusions were performed until the last day (16th) of digesta sampling. Before starting the ^{15}N infusion, a sample of the ruminal content was collected from each animal and stored at −20 °C for ^{15}N background determination in the ruminal digesta. These results are shown in a complementary study.

A total of eight omasal and ileal digesta samples were collected per animal from the 14th to the 16th d of each period, with 9 h of interval between them. Samplings were performed at 0600 and 1500 h on 14th d; on the 15th d at 0000, 0900, and 1800 h; and on the 16th d at 0300, 1300, and 2100 h, totaling eight samples of each digesta site (omasal or ileal), which were used to make a composite sample per animal. Omasal digesta sampling was performed according to Huhtanen et al. (1997) with adaptations described in Leão (2002). Sampling procedures were similar to the ones described by Mariz et al. (2013). To perform ileal sampling, plastic bags were placed at the end of the ileal cannula, allowing free digesta flow into the collection bags. After collection, all omasal and ileal digesta samples were immediately frozen at −80 °C and subsequently lyophilized (Freezemobile 24, Virtis, Gardiner, NY) and ground in a Wiley Mill (TE-648, TECNAL, Piracicaba, Brazil) with 2 and 1 mm sieves. The samples were stored for further chemical DM, CP, and AA analysis.

The procedure used for bacterial isolation was described by Reynal et al. (2005) with modifications described by Krizsan et al. (2010). Samples were taken at 0600, 1500, 0000, 0900, 1800, 1500, 1200, and 2100 h), and then a composite sample was pooled, resulting in 1.2 liters of omasal composite sample and 800 mL of ileal composite sample. The composite samples were filtered through a 100-µm nylon mesh filter with 44% of pore surface area (Sefar Nitex 100/44, Sefar, Thal, Switzerland) and the material that remained on the filter was washed with 800 mL of 0.90% (wt/vol) saline solution (NaCl). The material remaining on the filter was saved for isolation of particle-associated bacteria (**PAB**).

The filtrate material was centrifuged at 1,000 × g for 10 min at 5 °C for isolation of liquid-associated bacteria (**LAB**). Thereafter, the supernatant from each composite sample was transferred to tubes and re-centrifuged at 11,250 × g for 30 min at 5 °C, and the resulting supernatant was discarded. The remaining pellet was suspended in 20 mL of McDougall buffer (McDougall, 1948) and re-centrifuged at 16,250 × g for 20 min at 5 °C. The supernatant was discarded, and the pellet resulting from this centrifugation consisted of the LAB and was stored at −40 °C in aluminum trays for subsequent lyophilization. The solid material retained on the 100-µm filter and the pellets generated from the first centrifugation step of LAB isolation from

each steer were transferred to a 1,000-mL bottle, and 700 mL of 0.90% (wt/vol.) NaCl solution containing 0.1% (vol./vol.) of Tween 80 was added. The contents were homogenized and maintained at 4 °C overnight. On the next day of PAB isolation, the material was filtered through the 100-μm nylon mesh filter and the filtrate was subjected to the same centrifugation procedures used for LAB isolation. The supernatant was discarded and the pellet was stored at −40 °C. Pellets resulting from LAB and PAB isolation were stored at −80 °C; freeze dried (Freezemobile 24, Virtis, Gardiner, NY) and then macerated with a plastic pestle and mortar; and stored at −20 °C in plastic containers with a lid for further chemical DM, OM, CP, and ^{15}N analysis.

Experiment 2. Performance Trial

Animals, experimental design, diets, and carcass evaluation. Data from a complementary study (experiment 2) were used to calculate the efficiency of individual and total AA utilization for whole-body protein retention. A total of 44 bulls (22 Nellore and 22 crossbred F1 Angus × Nellore) with 8 months and initial shrunk BW (SBW) 215.0 ± 15.0 kg (Nellore = 208.0 ± 12.78 kg; Angus × Nellore = 221.9 ± 14.16 kg) were used in experiment 2. Eight of those animals (four from each genetic group) were slaughtered at the beginning of the experiment and were used as baseline to estimate initial body composition for all them. The remaining 36 bulls were randomly assigned to one of the three dietary treatments previously described for voluntary intake. This experiment was conducted in a completely randomized design with six replicates, in a 2 × 3 factorial scheme. The factors were two genetic groups (Nellore and crossbred F1 Angus × Nellore, A×N) and three CP contents (100, 120, and 140 g CP/kg DM). The experimental period lasted 224 d, and all animals were slaughtered at the end of the experiment to evaluate their AA body composition. The DMI was monitored daily, allowing 5% orts based on fresh feed.

After slaughtering, the carcass of each animal was divided into two halves that were weighed and cooled in a cold chamber at 4 °C for 24 h. After 24 h postmortem, the right half-carcass was dissected to separate the muscle plus fat from bones, and each portion was weighed. The muscle plus fat of each animal were ground and homogenized to obtain a composite sample of muscle and fat proportional to their natural weight in the empty BW. All samples were freeze dried and, subsequently, ground in an industrial

blender (Eberbach Model No. E8017.00) using liquid nitrogen to enable sample grinding since the components had not been partially degreased. The samples were stored at −20 °C for further chemical AA analysis. This experiment allowed accessing the daily total and individual AA retained in whole body per kg of DMI.

Experiments 1 and 2

Chemical analyses. Feeds, orts, feces, and omasal and ileal digesta samples were analyzed for DM, OM, and N according to methods 934.01, 942.05, and 968.06, respectively (AOAC, 2006). The EE content was analyzed according to method 920.39 (AOAC, 2006). NDF corrected for ash and protein ($_{ap}$NDF) was made adding thermostable α-amylase, without sodium sulfite addition, and the residues were analyzed for ash (Mertens, 2002) and N compounds (Licitra et al., 1996). The iNDF content was evaluated after in situ incubation of 2-mm ground samples for 288 h (Valente et al., 2015). Cobalt concentration was determined using an atomic absorption spectrophotometer (Spctr AA-800; Varian spectrometer, Harbor City, CA). The non-fibrous carbohydrates (**NFC**s) were calculated according to Detmann and Valadares Filho (2010), in which NFC = 100 − [(% CP − % urea CP + % urea) + %$_{ap}$NDF + % EE + % ash].

The ^{15}N analyses were performed according to Machado et al. (2013). The ^{15}N excess atoms were measured using an isotope ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany). Samples containing approximately 100 μg of N were weighed and placed in 5 to 8 mm capsules for future readings. The stable isotope rate of the same chemical element (^{15}N:^{14}N) was evaluated in terms of Δ per thousand, according to specific international standard.

Samples of feeds, omasal digesta, and ileal digesta; bacterial pool (LAB and PAB) from experiment 1; and composite sample of empty body weight, including blood, hide, head and hoofs, organs and viscera, muscle plus fat, and bones proportional to their natural weight, taken from experiment 2, were analyzed for AA contents. The determination of each AA concentration was carried out by HPLC. The methodology involves the reaction of the precolumn derivatization with phenylisothiocyanate to form the PTC AA, which were quantitated by HPLC in reverse phase (30 μl loop injection, pH 6.40, in a binary linear gradient with a flow of 1 mL/min and column temperature 58 °C) using UV detection at 254 nm.

Calculations

Nutrient flow. Marker concentrations at the different phases of the omasal digesta were used to estimate the DM flow through the reconstitution factor (France and Siddons, 1986). The ileal digesta flow of DM (g/d) was obtained by dividing the iNDF intake by its concentration in the ileal digesta sample. The flow of microbial N at the omasum or ileum was calculated by N flow from the respective sampling site multiplied by the ratio obtained with digesta ^{15}N enrichment:bacteria ^{15}N enrichment. The daily amounts (g/d) of individual AA absorbed were calculated by the difference between AA flow at the omasum and ileum. The flow (g/d) of each individual AA that passed by the omasum and ileum compartments was calculated by multiplying the respective concentration (g AA/kg DM) in the digesta sample by the DM flow from the respective sampling site. The flow (g/d) of each individual microbial AA that passed through the omasum and ileum compartments was calculated by multiplying the respective concentration (g AA/kg DM) of each AA in the isolated bacteria by microbial N flow in the respective sampling site.

Apparent digestibility. The apparent digestibility of total AA (individual, EAA, and nonessential AA [**NEAA**]) and microbial AA (individual, EAA, and NEAA) in SI was calculated by difference between AA flow in omasum and ileum flow, divided by AA omasum flow. True intestinal digestibility of total AA (individual, EAA, and NEAA) and microbial AA (individual, EAA, and NEAA) was estimated by linear regression model fitted between the AA absorbed in SI ($\hat{Y}$; g/d) and their respective omasal flow (X; g/d). The intercept of the equation represented the endogenous losses, and the slope represented the true digestibility of the total AA or microbial AA.

Whole-body AA deposition. The daily amount (g/d/SBW) of AA retained, from experiment 2, was calculated by the difference between each AA (g) in whole body of each slaughtered animal at the end of the experiment and their value at the beginning (estimated from mean value of AA [g] in whole body of reference animals), divided by days in feedlot.

The daily amount (g/d) of total and individual absorbed AA from experiment 1 was expressed by DMI of each cannulated animal (g/kg DMI) and a treatment mean was obtained. These means obtained from experiment 1 (g/kg DMI) for each treatment were multiplied by the DMI of each animal in experiment 2 of respective treatment, to estimate the daily amount (g/d) of AA absorbed for each experimental unit in experiment 2.

The efficiency of whole-body AA deposition was estimated by linear regression model, which was fitted between the total and individual AA

Table 2. Proportion of AA in the ingredients and in the diets (% DM)

Item	Ingredients				Dietary CP (g/kg DM)		
	CS	Corn grain	Wheat meal	Soybean meal	100	120	140
EAA							
Arginine	0.36	0.40	1.77	3.99	0.54	0.59	0.65
Histidine	0.07	0.18	0.61	1.32	0.17	0.19	0.21
Isoleucine	0.22	0.24	0.79	2.40	0.31	0.35	0.39
Leucine	0.49	0.90	1.54	4.09	0.80	0.86	0.93
Lysine	0.27	0.36	1.01	4.13	0.43	0.51	0.60
Methionine	0.05	0.13	0.26	0.53	0.11	0.11	0.12
Phenylalanine	0.21	0.34	0.98	2.68	0.36	0.40	0.45
Threonine	0.18	0.25	0.76	2.05	0.29	0.32	0.35
Tryptophan	0.03	0.13	0.20	0.36	0.21	0.21	0.22
Valine	0.28	0.31	1.04	2.11	0.37	0.40	0.43
NEAA							
Alanine	0.54	0.56	1.13	2.41	0.62	0.65	0.69
Aspartic	0.29	0.48	1.89	5.96	0.58	0.69	0.79
Cystine	0.04	0.08	0.27	0.49	0.08	0.09	0.09
Glutamine	0.62	1.37	4.35	9.46	1.72	1.84	1.97
Glycine	0.30	0.36	1.37	2.56	0.44	0.47	0.49
Proline	0.38	0.79	1.49	3.02	0.67	0.71	0.75
Serine	0.17	0.40	1.14	2.97	0.49	0.54	0.59
Tyrosine	0.12	0.22	0.69	1.77	0.23	0.58	0.62

Table 3. Effect of dietary CP content on total EAA and NEAA flow in omasum and ileum

Item	CP contents (g/kg DM)[1]				SEM	Contrast[2]		
	CR	100	120	140		RI vs. VI	Linear	Quadratic
Flow of AA (g/d)								
EAA omasum	160.8	264.0	288.4	345.9	0.02	<0.01	<0.01	0.38
NEAA omasum	189.5	313.7	353.0	409.7	0.03	<0.01	<0.01	0.69
EAA ileum	43.7	73.8	71.6	86.4	0.01	<0.01	0.10	0.19
NEAA ileum	56.8	97.1	93.7	115.2	0.01	<0.01	0.05	0.11
Flow of EAA in omasum (g/d)								
Arginine	16.49	26.79	29.80	34.70	1.77	<0.01	<0.01	0.63
Histidine	6.91	10.90	12.05	14.19	0.96	<0.01	0.01	0.65
Isoleucine	18.20	29.91	32.77	38.41	1.82	<0.01	<0.01	0.50
Leucine	31.98	53.05	58.51	68.59	3.59	<0.01	<0.01	0.55
Lysine	24.19	42.62	45.28	57.78	2.93	<0.01	<0.01	0.11
Methionine	6.66	10.72	12.02	14.09	0.69	<0.01	<0.01	0.64
Phenylalanine	16.63	26.92	29.04	35.64	1.93	<0.01	<0.01	0.33
Threonine	16.74	27.48	30.56	36.91	1.75	<0.01	<0.01	0.38
Tryptophan	2.73	4.24	5.12	5.58	0.39	<0.01	<0.01	0.56
Valine	20.32	31.37	33.19	39.95	2.74	<0.01	0.03	0.47
Flow of nonessential in omasum AA (%)								
Alanine	24.66	41.67	47.28	54.94	2.63	<0.01	<0.01	0.70
Aspartic acid	33.24	55.19	63.01	73.95	4.68	<0.01	0.01	0.78
Cystine	7.43	11.40	12.87	14.22	0.74	<0.01	0.01	0.95
Glutamic acid	30.38	58.05	55.60	70.26	4.91	<0.01	0.05	0.11
Glycine	20.62	34.86	39.78	45.67	2.32	<0.01	<0.01	0.83
Proline	19.10	33.66	38.56	42.87	2.18	<0.01	<0.01	0.89
Serine	17.33	28.27	31.91	37.95	1.87	<0.01	<0.01	0.54
Tyrosine	17.23	27.09	29.51	35.88	1.74	<0.01	<0.01	0.34
Flow of EAA in ileum (g/d)								
Arginine	3.82	6.26	6.04	7.37	0.54	<0.01	0.07	0.14
Histidine	1.48	2.61	2.39	2.98	0.19	<0.01	0.11	0.05
Isoleucine	5.11	8.09	8.23	9.75	0.72	<0.01	0.04	0.30
Leucine	8.17	13.90	13.41	15.74	1.10	<0.01	0.16	0.21
Lysine	6.71	12.52	12.01	14.32	1.69	<0.01	0.40	0.44
Methionine	1.18	2.08	1.98	2.29	0.17	<0.01	0.32	0.25
Phenylalanine	4.55	7.42	7.42	8.97	0.61	<0.01	0.03	0.19
Threonine	5.99	9.95	9.54	11.98	0.80	<0.01	0.06	0.12
Tryptophan	0.88	1.55	1.32	1.67	0.14	<0.01	0.41	0.03
Valine	5.82	9.40	9.30	11.29	0.84	<0.01	0.05	0.19
Flow of nonessential in ileum AA (%)								
Alanine	8.10	13.54	13.33	16.04	1.08	<0.01	0.06	0.19
Aspartic acid	6.99	12.05	11.78	16.16	1.65	<0.01	0.01	0.10
Cystine	1.84	2.98	3.29	3.35	0.39	<0.01	0.41	0.76
Glutamic acid	13.97	24.05	22.73	28.37	2.05	<0.01	0.06	0.07
Glycine	8.07	13.61	13.12	15.63	0.94	<0.01	0.08	0.13
Proline	7.10	12.35	11.61	13.49	0.89	<0.01	0.28	0.16
Serine	5.19	9.16	8.50	10.47	0.66	<0.01	0.14	0.09
Tyrosine	5.50	9.36	9.34	11.66	0.93	<0.01	0.02	0.15

[1]Treatments: RI = restricted intake to 1.2% BW containing 120 g of CP/kg of DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g of CP/kg of DM, respectively.

[2]RI vs. VI = restricted intake vs. voluntary intake (100, 120, and 140 CP g of CP/kg of DM).

absorbed in SI ($\hat{Y}$; g/d) and their respective whole-body retention (X; g/d). The intercept of the equation represented the endogenous losses, and the slope represented the AA retention efficiency.

Statistical Analyses

The variables regarding AA intake and apparent digestibility were analyzed using the MIXED

procedure of SAS software (version 9.1) using the following statistical model:

$$Y_{ijkl} = \mu + q_i + N_j + QN_{ij} + a_{(i)k} + P_{(i)l} + \varepsilon_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = general mean, q_i = random effect of the Latin square/genetic group i, N_j = fixed effect of the dietary CP content j, qN_{ij} = random effect of the interaction between Latin square i and the dietary CP content j, $a_{(i)k}$ = random effect of animal K nested in the Latin square i, $p_{(i)l}$ = random effect of period 1 nested in the Latin square i, and ε_{ijkl} = random error taken as normal and independently distributed (NID) $(0; \sigma^2_\varepsilon)$.

The degrees of freedom were estimated by the Kenward–Roger method. In the case of significant effects /for CP content, the following orthogonal contrasts were studied: C1: restricted intake vs. voluntary intake; C2: linear effect of CP dietary contents 100, 120, and 140 g/kg; and C3: quadratic effect for the same increasing contents. All the statistical procedures were carried out using 0.05 as critical level of probability p for type 1 error.

True intestinal digestibilities of AA were estimated by linear regression model. The adjusted model for the true digestibility of the microbial AA took into consideration the effect of the microbial markers on the obtained estimates, using this factor as a Dummy variable. Animal and period of Latin square were used as random variables. In case of similarity between the estimates, a single regression equation was adjusted for the data described above. The data were analyzed using PROC GLM of SAS (version 9.2) and 0.05 as critical level for probability of type 1 error, then P-values lesser than 0.05 were considered significant. The P-values between 0.05 and 0.10 were considered a significant trend. Same procedure was performed for efficiency of individual and total AA utilization from absorption to whole-body protein deposition.

RESULTS

Flow of AA and EAA

The total and individual EAA ($P < 0.01$) and NEAA ($P < 0.01$) flow to the omasum increased linearly in response to dietary CP content (Table 3). However, RI showed lower ($P < 0.01$) flow of total AA (g/d) in relation to voluntary intake. There was a greater ($P < 0.01$) individual AA flow at the SI for voluntary intake (**VI**) than RI.

Table 4. Effect of dietary CP content on EAA digestibility

Item	CP contents (g/kg DM)[1]					Contrast[2]		
	CR	100	120	140	SEM	RI vs. VI	Linear	Quadratic
Apparent absorption of EAA (g/d)								
Arginine	12.7	20.5	23.8	27.3	0.002	<0.01	<0.01	0.91
Histidine	5.4	8.3	9.7	11.2	0.001	<0.01	0.02	0.91
Isoleucine	13.1	21.8	24.5	28.7	0.002	<0.01	<0.01	0.67
Leucine	23.8	39.1	45.1	52.9	0.004	<0.01	<0.01	0.78
Lysine	17.5	30.1	33.3	43.5	0.004	<0.01	<0.01	0.21
Methionine	5.5	8.6	10.1	11.8	0.001	<0.01	<0.01	0.83
Phenylalanine	12.1	19.5	21.6	26.7	0.002	<0.01	<0.01	0.45
Threonine	10.7	17.5	21.0	24.9	0.002	<0.01	<0.01	0.87
Tryptophan	1.9	2.7	3.8	3.92	0.001	<0.01	<0.01	0.12
Valine	14.5	22.0	24.0	28.7	0.003	<0.01	0.05	0.61
Apparent digestibility of EAA (%)								
Arginine	76.6	76.4	79.7	78.9	1.76	0.16	0.10	0.11
Histidine	78.0	75.1	80.4	78.7	2.05	0.93	0.07	0.05
Isoleucine	72.0	72.8	74.8	74.8	1.94	0.07	0.15	0.39
Leucine	74.2	73.5	77.1	77.1	1.85	0.13	0.01	0.14
Lysine	71.1	71.1	73.1	75.1	4.41	0.49	0.26	0.99
Methionine	81.9	80.5	83.4	83.5	1.78	0.61	0.05	0.26
Phenylalanine	72.5	71.9	74.5	74.7	2.13	0.44	0.13	0.43
Threonine	64.1	63.7	68.3	67.4	2.87	0.21	0.12	0.18
Tryptophan	68.7	61.9	73.1	69.2	5.16	0.83	0.07	0.03
Valine	70.9	68.7	72.3	71.5	2.76	0.95	0.28	0.33
Total EAA	72.7	71.8	75.1	75.1	0.02	0.34	0.07	0.27

[1]Treatments: RI = restricted intake to 1.2% BW containing 120 g of CP/kg of DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g of CP/kg of DM, respectively.

[2]RI vs. VI = restricted intake vs. voluntary intake (100, 120, and 140 CP g of CP/kg of DM).

Table 5. Effect of dietary CP content on NEAA digestibility

Item	CP contents (g/kg of DM)[1]				SEM	Contrast[2]		
	CR	100	120	140		RI vs. VI	Linear	Quadratic
Apparent absorption of NEAA (g/d)								
Alanine	16.6	28.2	34.0	38.9	0.003	<0.01	<0.01	0.81
Aspartic acid	26.3	43.1	51.2	57.8	0.01	<0.01	0.01	0.87
Cystine	5.2	7.2	70	8.5	1.81	<0.01	0.13	0.25
Glutamic acid	36.3	58.7	70.0	78.3	0.01	<0.01	<0.01	0.78
Glycine	12.5	21.3	26.7	30.0	0.003	<0.01	<0.01	0.54
Proline	12.0	21.3	27.0	29.4	0.002	<0.01	<0.01	0.34
Serine	12.2	19.1	23.4	27.5	0.002	<0.01	<0.01	0.92
Tyrosine	11.7	17.7	20.2	24.2	0.002	<0.01	<0.01	0.60
Apparent digestibility of NEAA (%)								
Alanine	66.8	67.5	71.5	70.8	2.35	0.01	0.02	0.05
Aspartic acid	79.1	77.4	81.2	77.6	3.39	0.84	0.93	0.07
Cystine	68.7	64.1	61.1	68.0	11.08	0.34	0.48	0.30
Glutamic acid	72.3	70.7	75.6	73.3	2.12	0.50	0.12	0.02
Glycine	60.6	60.9	66.1	65.6	2.83	0.01	0.01	0.05
Proline	62.3	63.4	69.4	68.5	2.60	<0.01	<0.01	0.02
Serine	69.8	67.6	73.1	72.3	2.26	0.41	0.01	0.04
Tyrosine	68.3	65.3	68.5	67.6	2.68	0.51	0.28	0.26
Total NEAA	68.9	73.4	71.9	70.0	0.02	0.27	0.06	0.03
Total AA								
Ap.Ab[3] (g/d)	249.8	406.8	476.0	554.0	0.04	<0.01	<0.01	0.89
AD[4] (%)	71.2	70.2	74.2	73.4	0.02	0.30	0.06	0.10

[1]Treatments: RI = restricted intake to 1.2% BW containing 120 g of CP/kg of DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g of CP/kg of DM, respectively.

[2]RI vs. VI = restricted intake vs. voluntary intake (100, 120, and 140 CP g of CP/kg of DM).

[3]Ap.Ab = apparent absorption.

[4]AD = apparent digestibility.

There was a greater ($P < 0.01$) absorption (g/d) of individual EAA in the SI for voluntary intake than RI (Table 4). Similar to the total AA flow, there was a linear effect ($P < 0.05$) in amounts of EAA absorbed in the SI in response to CP content. The apparent digestibility did not differ ($P > 0.05$) between RI and VI, with exception for isoleucine ($P = 0.07$). The apparent digestibility of EAA was not affected ($P > 0.05$) by CP content, with exception for leucine ($P = 0.01$, linear effect) and methionine ($P = 0.05$, linear effect). The apparent digestibility of histidine ($P = 0.05$) and tryptophan ($P = 0.03$) showed quadratical effect in response to CP content.

Nonessential AA

Animals in VI had a greater ($P < 0.01$) absorption of individual NEAA compared with animals fed RI (Table 5). As expected, the absorption (g/d) of the NEAA in SI increased linearly ($P < 0.05$), with exception of cystine ($P = 0.13$), in response to increasing dietary CP. However, difference was observed among AA when the apparent

Table 6. True intestinal digestibility of AA

Item	Equations	Slope P-value
Total AA	$\hat{Y} = -17.18_{(\pm15.44)} + 0.75_{(\pm0.02)} X$	<0.01
EAA	$\hat{Y} = -8.14_{(\pm7.21)} + 0.77_{(\pm0.24)} X$	<0.01
NEAA	$\hat{Y} = -9.29_{(\pm8.56)} + 0.74_{(\pm0.02)} X$	<0.01
EAA		
Arginine	$\hat{Y} = -0.76_{(\pm0.59)} + 0.81_{(\pm0.01)} X$	<0.01
Histidine	$\hat{Y} = -0.47_{(\pm0.30)} + 0.83_{(\pm0.02)} X$	<0.01
Isoleucine	$\hat{Y} = -0.87_{(\pm0.68)} + 0.77_{(\pm0.02)} X$	<0.01
Leucine	$\hat{Y} = -1.78_{(\pm1.15)} + 0.79_{(\pm0.01)} X$	<0.01
Lysine	$\hat{Y} = -1.70_{(\pm2.26)} + 0.77_{(\pm0.05)} X$	<0.01
Methionine	$\hat{Y} = -0.38_{(\pm0.25)} + 0.86_{(\pm0.02)} X$	<0.01
Phenylalanine	$\hat{Y} = -1.21_{(\pm0.68)} + 0.78_{(\pm0.02)} X$	<0.01
Threonine	$\hat{Y} = -1.65_{(\pm1.04)} + 0.72_{(\pm0.03)} X$	<0.01
Tryptophan	$\hat{Y} = -0.56_{(\pm0.22)} + 0.82_{(\pm0.04)} X$	<0.01
Valine	$\hat{Y} = -1.69_{(\pm0.94)} + 0.76_{(\pm0.02)} X$	<0.01
NEAA		
Alanine	$\hat{Y} = -1.97_{(\pm1.16)} + 0.74_{(\pm0.02)} X$	<0.01
Aspartic acid	$\hat{Y} = -1.08_{(\pm1.83)} + 0.81_{(\pm0.02)} X$	<0.01
Cystine	$\hat{Y} = -1.41_{(\pm0.70)} + 0.85_{(\pm0.06)} X$	<0.01
Glutamic acid	$\hat{Y} = -1.91_{(\pm2.13)} + 0.75_{(\pm0.02)} X$	<0.01
Glycine	$\hat{Y} = -2.59_{(\pm1.18)} + 0.71_{(\pm0.02)} X$	<0.01
Proline	$\hat{Y} = -2.23_{(\pm1.05)} + 0.73_{(\pm0.02)} X$	<0.01
Serine	$\hat{Y} = -1.30_{(\pm0.87)} + 0.76_{(\pm0.02)} X$	<0.01
Tyrosine	$\hat{Y} = -0.05_{(\pm0.89)} + 0.67_{(\pm0.02)} X$	<0.01

Table 7. Effect of dietary CP content on flow of microbial AA at the omasum

Item	CP contents (g/kg DM)[1]					Contrast[2]		
	CR	100	120	140	SEM	RI vs. VI	Linear	Quadratic
Flow of AA (g/d)								
EAA omasum	151.9	179.8	206.2	237.9	17.15	<0.01	0.01	0.87
NEAA omasum	174.3	214.2	242.7	277.8	19.00	<0.01	0.02	0.87
EAA ileum	32.3	49.3	49.6	52.1	5.80	0.01	0.71	0.86
NEAA ileum	39.8	61.7	60.9	65.6	7.26	0.01	0.69	0.74
Flow of EAA (g/d)								
Arginine	16.33	19.80	22.39	25.08	1.98	0.01	0.05	0.98
Histidine	7.12	8.27	9.42	10.37	0.88	0.02	0.05	0.91
Isoleucine	17.91	20.84	24.22	28.37	1.98	<0.01	0.01	0.84
Leucine	28.52	33.85	39.35	45.23	3.18	<0.01	0.01	0.95
Lysine	22.39	27.11	30.23	34.56	3.17	0.03	0.08	0.85
Methionine	8.58	10.14	11.77	13.54	1.04	0.01	0.03	0.95
Phenylalanine	15.51	18.64	21.38	25.51	1.72	<0.01	0.01	0.69
Threonine	17.55	20.64	23.62	27.39	1.88	<0.01	0.01	0.84
Valine	18.03	20.57	23.83	27.86	1.91	<0.01	<0.01	0.82
Flow of NEAA (%)								
Alanine	24.17	28.85	33.06	37.52	2.70	0.01	0.03	0.97
Aspartic acid	35.40	46.47	50.35	60.73	3.82	<0.01	0.02	0.48
Cystine	4.22	5.01	5.44	6.33	0.43	0.01	0.03	0.63
Glutamic acid	43.30	52.50	60.10	69.50	4.74	0.01	0.02	0.87
Glycine	21.71	26.42	30.47	33.41	2.46	0.01	0.06	0.85
Proline	14.36	17.38	20.07	22.65	1.78	0.02	0.06	0.98
Serine	15.38	18.71	21.07	24.36	1.70	<0.01	0.02	0.80
Tyrosine	15.79	18.88	22.09	23.36	1.85	<0.01	0.05	0.59

[1]Treatments: RI = restricted intake to 1.2% BW containing 120 g of CP/kg of DM; 100, 120 and 140 = voluntary intake containing 100, 120 and 140 g of CP/kg of DM, respectively.

[2]RI vs. VI = restricted intake vs. voluntary intake (100, 120, and 140 CP g of CP/kg of DM).

digestibility (%) of all NEAA was compared. The apparent digestibility of alanine ($P = 0.05$), aspartic acid ($P = 0.07$), glutamic acid ($P = 0.02$), glycine ($P = 0.05$), proline ($P = 0.02$), and serine ($P = 0.04$) responded quadratically to CP increase. However, the apparent digestibilities of cystine ($P = 0.30$) and tyrosine ($P = 0.26$) were not affected by increasing dietary CP.

True Intestinal Digestibility of AA

Our estimates of true intestinal digestibility were obtained by regression analysis (Table 6). For total AA, EAA, and NEAA, the estimated endogenous losses and true digestibility were 17.2 g/d and 75%; 8.1g/d and 77%; and 9.3 g/d and 74%, respectively. True digestibility of individual EAA varied from 72% for threonine to 86% to methionine, while it varied from 67% for tyrosine to 85% to cystine for NEAA. The true intestinal digestibility of lysine was 77% with 1.7 g of endogenous losses. The methionine had

greater (86%) true intestinal digestibility in comparison to other EAA.

Microbial EAA and NEAA

As expected, total microbial EAA ($P = 0.01$) and NEAA ($P = 0.02$) flowing to the omasum increased linearly with CP content (Table 7). The individual flow of microbial AA increased linearly ($P < 0.05$) in response to increasing dietary CP.

The RI showed lower ($P < 0.01$) absorption of individual EAA in the SI compared with voluntary intake (Table 8). The individual amounts of microbial AA increased linearly ($P < 0.05$) in response to increasing dietary CP. The intestinal digestibility was similar ($P > 0.05$) between RI and VI. The CP content did not affect ($P > 0.05$) the intestinal digestibility of the individual microbial AA, with exception for isoleucine ($P = 0.08$, linear effect), leucine ($P = 0.05$, linear effect), and phenylalanine ($P = 0.09$, linear effect), which responded linearly.

Table 8. Effect of dietary CP content on essential microbial AA digestibility

Item	CP contents (g/kg of DM)[1]				SEM	Contrast[2]		
	CR	100	120	140		RI vs. VI	Linear	Quadratic
Apparent absorption of essential microbial AA (g/d)								
Arginine	13.1	14.8	17.6	19.9	1.87	0.04	0.05	0.90
Histidine	5.3	5.6	6.8	7.7	0.79	0.07	0.03	0.83
Isoleucine	13.8	14.8	17.8	22.0	1.89	0.03	0.01	0.74
Leucine	22.3	23.9	29.8	35.4	2.99	0.04	0.01	0.96
Lysine	19.0	21.4	24.8	28.5	2.93	0.05	0.06	0.95
Methionine	6.8	7.4	8.9	10.5	0.93	0.05	0.02	0.98
Phenylalanine	11.8	12.9	15.7	19.4	1.64	0.02	0.01	0.77
Threonine	13.7	14.9	17.7	21.1	1.68	0.03	0.01	0.89
Tryptophan	–	–	–	–	–	–	–	–
Valine	13.9	14.9	17.5	21.3	1.80	0.03	0.01	0.72
Apparent digestibility of essential microbial AA (%)								
Arginine	80.0	74.6	78.2	78.9	2.50	0.33	0.23	0.62
Histidine	74.5	67.4	71.8	73.7	3.13	0.30	0.14	0.73
Isoleucine	76.9	70.6	73.2	77.2	2.93	0.28	0.08	0.82
Leucine	78.1	70.3	75.4	77.9	2.63	0.22	0.05	0.67
Lysine	81.5	82.3	84.8	78.3	2.20	0.10	0.18	0.62
Methionine	79.1	73.2	75.9	77.2	2.84	0.24	0.30	0.83
Phenylalanine	76.0	69.0	72.9	75.9	2.85	0.28	0.09	0.88
Threonine	78.5	71.9	74.8	76.8	2.60	0.16	0.15	0.87
Tryptophan	–	–	–	–	–	–	–	–
Valine	77.2	72.0	73.0	76.0	2.98	0.27	0.30	0.76
Total EAA microbial	78.8	72.3	75.6	77.8	2.57	0.20	0.12	0.84

[1]Treatments: RI = restricted intake to 1.2% BW containing 120 g of CP/kg of DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g of CP/kg of DM, respectively.

[2]RI vs. VI = restricted intake vs. voluntary intake (100, 120, and 140 CP g of CP/kg of DM).

The individual amounts of microbial NEAA were different ($P < 0.05$) between RI and VI, with exception for cystine ($P = 0.16$) (Table 9). Also, an increase in CP content promoted a linear effect ($P < 0.05$) on individual amounts of microbial NEAA absorbed, except for cystine ($P = 0.14$). The apparent digestibility of microbial NEAA was similar ($P > 0.05$) between animals fed in RI or VI. In addition, the apparent digestibility did not differ ($P > 0.10$) when increasing dietary CP content, except for glycine ($P = 0.07$, linear tendency), proline ($P = 0.04$, linear tendency), and serine ($P = 0.07$, linear tendency).

True Intestinal Digestibility of Microbial AA

The true intestinal digestibility of total microbial AA was 80%, and the endogenous loss was 21.3 g/d (Table 10). The microbial arginine showed higher value for true intestinal digestibility (85%) in comparison to other microbial EAA. The estimated endogenous losses and intestinal digestibilities of microbial lysine and methionine were 0.6 g/d and 84%; 0.4 g/d and 80%, respectively.

Efficiency of AA Utilization

The efficiency of utilization of total AA obtained in this study was 40% (Table 11). The efficiencies of utilization of EAA and NEAA were 38% and 42%, respectively. The efficiencies of utilization of lysine and methionine were 37% and 58%, respectively. For EAA, the arginine showed greater efficiency of utilization (56%) compared with other AA. The efficiency of glutamic acid utilization was 67%; this value was greater in comparison to other NEAA.

DISCUSSION

The supply of AA reaching the SI determines whether or not the protein requirement of the ruminant is met (Shabi et al., 2000). In this way, we can infer that converting dietary CP to body protein is influenced by AA profile that reaches the SI, as well as AA digestibility. In this study, increased dietary CP content resulted in greater amounts of total AA that reaches the SI, which is consistent with our expectation and previously reported studies (Van Bruchem et al., 1989; Wang et al., 2016).

Table 9. Effect of dietary CP content on nonessential microbial AA and digestibility

Item[1]	CP contents (g/kg of DM)[2]					Contrast[3]		
	CR	100	120	140	SEM	RI vs. VI	Linear	Quadratic
Apparent absorption of nonessential microbial AA (g/d)								
Alanine	17.7	18.7	22.9	26.7	2.50	0.08	0.03	0.93
Aspartic acid	28.4	36.4	39.8	48.8	3.43	0.01	0.03	0.51
Cystine	2.7	3.0	3.4	4.0	0.44	0.16	0.14	0.81
Glutamic acid	31.7	34.6	42.0	50.3	4.32	0.04	0.02	0.94
Glycine	17.2	18.9	23.5	26.0	2.23	0.05	0.04	0.70
Proline	10.9	11.6	15.0	17.4	1.74	0.08	0.04	0.82
Serine	12.3	13.6	16.3	19.3	1.58	0.03	0.01	0.96
Tyrosine	13.6	15.7	18.8	19.9	1.82	0.02	0.07	0.59
Apparent digestibility of nonessential microbial AA (%)								
Alanine	73.4	64.7	69.1	70.4	3.44	0.16	0.21	0.68
Aspartic acid	80.4	78.3	79.0	80.2	2.55	0.67	0.60	0.93
Cystine	64.3	60.0	61.7	61.9	5.13	0.61	0.80	0.90
Glutamic acid	73.2	65.8	69.6	72.0	3.27	0.29	0.20	0.86
Glycine	79.1	71.1	76.7	77.6	2.45	0.17	0.07	0.43
Proline	76.2	65.9	74.3	76.6	3.42	0.32	0.04	0.46
Serine	80.0	72.0	77.3	78.9	2.49	0.18	0.07	0.55
Tyrosine	86.4	83.0	84.7	84.8	2.19	0.38	0.55	0.78
Total NE micAA[1]	77.3	71.0	74.6	76.1	2.64	0.27	0.18	0.74
AA total								
Ap.Ab.[4] (g/d)	254.1	283.1	338.3	398.1	32.88	0.02	0.01	0.94
AD[5] (%)	78.0	71.6	75.1	76.9	2.60	0.24	0.15	0.78

[1]Total NE micAA = total nonessential microbial AA.

[2]Treatments: RI = restricted intake to 1.2% BW containing 120 g of CP/kg of DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g of CP/kg of DM, respectively.

[3]RI vs. VI = restricted intake vs. voluntary intake (100, 120, and 140 CP g of CP/kg of DM).

[4]Ap.Ab = apparent absorption.

[5]AD = apparent digestibility.

The flow of total AA increased approximately 31% and 20% when comparing the 140 g CP/kg DM diet with the 100 and 120 g CP/kg DM diets, respectively, which explains the greater amounts of total AA absorbed in the SI.

However, it is important to note that CP excess in beef cattle diets is associated with excessive N losses, due to unbalanced AA profile and/or excessive intestinal AA absorption (Robinson, 1997). When optimal EAA profiles are absorbed in SI as required by the animal, their efficiency of use for protein synthesis and deposition is maximized and the requirement for total absorbed AA is reduced to a minimum, and consequently, the urinary N excretion is lessened (Schwab et al., 2003). Based on the results of a complementary study, Mariz (2016) demonstrated that N excretions obtained with 100 and 120 g CP/kg DM diets were reduced for 39.7 and 29.9 g/d in relation to 140 g CP/kg DM diet, respectively. In addition, Amaral et al. (2018) verified that animals fed with 120 g CP/kg DM diet had a greater retention of these EAA on their carcasses compared with others fed with 100 or 140 g CP/kg DM diets;

and that 120 and 140 g CP/kg DM diets resulted in similar animal performance. Based on these results, we can infer that the 120 g CP/kg DM diet could provide an adequate amount of absorbed AA in the SI compared with 100 and 140 g CP/kg DM diets.

The EAA (histidine, methionine, and tryptophan) and NEEA (cystine) showed lower values for amount absorbed in SI compared with the other AA. The lower supply of absorbed AA in the SI has been associated mainly with extensive ruminal degradation of AA and to a lesser extent to intestinal digestion (Mijoun et al., 2010). Gao et al. (2016) reported that histidine was extensively degraded by rumen microorganisms for all the mixed diets; its average ruminal degradability was beyond 80%, indicating that intestinal supply of histidine might be inadequate for absorption. The extent of AA degradation may vary in the rumen, and then the AA profile of the absorbed fraction in the SI can be markedly different from that of the original feed (Erasmus et al., 1994). For this reason, accurately AA estimates that reach the SI is a challenging task that needs to be achieved (Mijoun et al., 2010).

Table 10. True intestinal digestibility of microbial AA

Item	Equations	Slope P-value
Total AA	$\hat{Y} = -21.27_{(\pm 29.90)} + 0.80_{(\pm 0.06)}X$	<0.01
EAA	$\hat{Y} = -10.87_{(\pm 13.57)} + 0.82_{(\pm 0.06)}X$	<0.01
NEAA	$\hat{Y} = -10.62_{(\pm 16.31)} + 0.79_{(\pm 0.06)}X$	<0.01
EAA		
Arginine	$\hat{Y} = -1.34_{(\pm 1.38)} + 0.85_{(\pm 0.06)}X$	<0.01
Histidine	$\hat{Y} = -0.60_{(\pm 0.82)} + 0.79_{(\pm 0.09)}X$	<0.01
Isoleucine	$\hat{Y} = -1.97_{(\pm 1.65)} + 0.83_{(\pm 0.06)}X$	<0.01
Leucine	$\hat{Y} = -3.19_{(\pm 2.53)} + 0.84_{(\pm 0.06)}X$	<0.01
Lysine	$\hat{Y} = -0.60_{(\pm 1.69)} + 0.84_{(\pm 0.05)}X$	<0.01
Methionine	$\hat{Y} = -0.36_{(\pm 0.78)} + 0.80_{(\pm 0.06)}X$	<0.01
Phenylalanine	$\hat{Y} = -1.60_{(\pm 1.52)} + 0.81_{(\pm 0.07)}X$	<0.01
Threonine	$\hat{Y} = -0.67_{(\pm 1.55)} + 0.79_{(\pm 0.06)}X$	<0.01
Tryptophan	–	–
Valine	$\hat{Y} = -1.03_{(\pm 1.75)} + 0.79_{(\pm 0.07)}X$	<0.01
NEAA		
Alanine	$\hat{Y} = -1.62_{(\pm 2.79)} + 0.75_{(\pm 0.08)}X$	<0.01
Aspartic acid	$\hat{Y} = -0.79_{(\pm 3.03)} + 0.81_{(\pm 0.06)}X$	<0.01
Cystine	$\hat{Y} = -0.43_{(\pm 0.69)} + 0.70_{(\pm 0.12)}X$	<0.01
Glutamic acid	$\hat{Y} = -3.20_{(\pm 4.90)} + 0.76_{(\pm 0.08)}X$	<0.01
Glycine	$\hat{Y} = -1.55_{(\pm 1.90)} + 0.81_{(\pm 0.06)}X$	<0.01
Proline	$\hat{Y} = -2.54_{(\pm 1.47)} + 0.87_{(\pm 0.07)}X$	<0.01
Serine	$\hat{Y} = -1.21_{(\pm 1.36)} + 0.83_{(\pm 0.06)}X$	<0.01
Tyrosine	$\hat{Y} = -0.87_{(\pm 1.15)} + 0.89_{(\pm 0.05)}X$	<0.01

Table 11. The efficiency of individual and total absorbed AA to whole-body protein deposition

Item	Equations	Slope P-value
Total AA	$\hat{Y} = -36.67_{(\pm 12.14)} + 0.40_{(\pm 0.02)}X$	<0.01
EAA	$\hat{Y} = -17.43_{(\pm 5.44)} + 0.38_{(\pm 0.02)}X$	<0.01
NEAA	$\hat{Y} = -18.80_{(\pm 7.57)} + 0.42_{(\pm 0.03)}X$	<0.01
EAA		
Arginine	$\hat{Y} = -2.17_{(\pm 1.01)} + 0.56_{(\pm 0.03)}X$	<0.01
Histidine	$\hat{Y} = -1.42_{(\pm 0.47)} + 0.62_{(\pm 0.04)}X$	<0.01
Isoleucine	$\hat{Y} = -1.41_{(\pm 0.61)} + 0.30_{(\pm 0.02)}X$	<0.01
Leucine	$\hat{Y} = -3.36_{(\pm 0.97)} + 0.30_{(\pm 0.01)}X$	<0.01
Lysine	$\hat{Y} = -2.28_{(\pm 1.04)} + 0.37_{(\pm 0.02)}X$	<0.01
Methionine	$\hat{Y} = -0.37_{(\pm 0.43)} + 0.58_{(\pm 0.03)}X$	<0.01
Phenylalanine	$\hat{Y} = -1.57_{(\pm 0.53)} + 0.31_{(\pm 0.02)}X$	<0.01
Threonine	$\hat{Y} = -1.42_{(\pm 0.54)} + 0.34_{(\pm 0.02)}X$	<0.01
Tryptophan	$\hat{Y} = -0.18_{(\pm 0.15)} + 0.39_{(\pm 0.04)}X$	<0.01
Valine	$\hat{Y} = -2.55_{(\pm 0.82)} + 0.40_{(\pm 0.03)}X$	<0.01
NEAA		
Alanine	$\hat{Y} = -1.25_{(\pm 1.11)} + 0.35_{(\pm 0.03)}X$	<0.01
Aspartic acid	$\hat{Y} = -3.65_{(\pm 2.10)} + 0.26_{(\pm 0.03)}X$	<0.01
Cystine	$\hat{Y} = -0.14_{(\pm 0.26)} + 0.18_{(\pm 0.02)}X$	<0.01
Glutamic acid	$\hat{Y} = -4.57_{(\pm 2.29)} + 0.67_{(\pm 0.05)}X$	<0.01
Glycine	$\hat{Y} = -0.68_{(\pm 2.51)} + 0.63_{(\pm 0.08)}X$	<0.01
Proline	$\hat{Y} = -0.40_{(\pm 1.53)} + 0.43_{(\pm 0.05)}X$	<0.01
Serine	$\hat{Y} = -1.16_{(\pm 0.60)} + 0.31_{(\pm 0.02)}X$	<0.01
Tyrosine	$\hat{Y} = -0.88_{(\pm 0.49)} + 0.26_{(\pm 0.02)}X$	<0.01

The similar apparent digestibility of individual AA was obtained in response to the increasing dietary CP content; however, this value varied widely depending on the specific AA. Despite the low amount of histidine and methionine absorbed in SI, these AA and other ones like arginine appeared to have greater apparent digestibility compared with all of AA evaluated. On average, the apparent digestibilities of the arginine, histidine, and methionine were 78.3%, 78.0%, and 82.4%, respectively. This demonstrated that the lower amount of absorbed AA was compensated by increasing in intestinal digestibility coefficients. Van Bruchem et al. (1989) showed that varying CP content and rumen protein degradability, true digestibility of individual AA in SI varied from 72% to 92%, increasing with the protein amount that reaches the duodenum.

The EAA and NEAA true digestibility varies widely depending on the specific AA. In particular, methionine exhibited greater intestinal digestibility (86%) compared with the other EAA. This is important because the supply of AA to the ruminant animal is a product of the amount of AA reaching the SI and its digestibility at this digestive site (Tigmeyer et al., 1990). Additionally, methionine and lysine are considered limiting AA for growing cattle (Titgemeyer et al., 1990; Batista et al., 2016). Lysine had the value of 77% for true digestibility. In

this study, the differences obtained for intestinal digestibility among EAA and NEAA suggested that individual AA digestibility should be considered in feed formulation systems.

As expected, the amounts of absorbed microbial AA in the SI increased as dietary CP increased as well. It is recognized that the microbial protein provides most of the AA that pass into the SI of ruminants (Clark et al., 1992). Our data indicate that bacterial AA contributed with approximately 70% of total absorbed AA in SI. Additionally, it is assumed that the AA pattern of microbial protein is fairly constant and similar to that of lean body, and not influenced by dietary changes (Schwab et al., 2003). The total amount of intestinal absorbable AA observed in this trial may indicate that arginine, isoleucine, leucine, lysine, phenylalanine, threonine, and valine can be greater in bacterial composition, while histidine and methionine appear to be lower.

In addition, our results showed that true intestinal digestibilities of total microbial AA were similar to the value of 80% adopted for microbial protein in some feeding systems (NRC, 1985; Valadares Filho et al., 2016); however, when considering true intestinal digestibility of each AA, these values can vary substantially (Storm et al., 1983; Fonseca et al., 2014). The different techniques

utilized and the difficulty to measure post-ruminal AA digestibility can explain these differences in the literature. Thus, new studies need to be conducted to enhance the understanding of the rumen microbial AA digestibility, and consequently improves the estimation of metabolizable protein in feed systems.

The requirement for absorbed protein can be determined by correcting the sum of the net protein requirements for maintenance and production with the efficiency with this absorbed AA are retained into tissue protein (NRC, 1985). In this trial, a difference was found for retention efficiency of total and individual AA. For EAA, arginine (56%), histidine (62%), and methionine (58%) showed the greater estimates for retention efficiency. For NEAA, glutamic acid (67%) and glycine (63%) showed the greater estimates for retention efficiency. This discrepancy among AA may suggest that there are differences in how efficiently they are used by growing cattle (Batista et al., 2016). Theoretically, the AA present in lowest amount relative to the animal requirement is used most efficiently (NRC, 1985). In addition, the value of 40% obtained in this study for efficiency of total absorbed AA is lower than the value of 47.4% recommended by the Brazilian system, and 49.2% recommended by the American systemNASEM (2016). Results from this study indicate that there is a need for additional research to derive more accurate estimates of efficiency of AA utilization in beef cattle.

CONCLUSIONS

The increase in dietary CP content results in greater flows of total AA to SI. The CP content had little effect on intestinal AA apparently digestibility of most AA; however, these values varied widely depending on the specific AA. The true intestinal digestibilities of lysine and methionine were 77% and 86%, respectively. The true intestinal digestibilities of total microbial AA, microbial lysine, and methionine were 80%, 84%, and 80%, respectively.

The efficiency of total AA utilization for whole-body protein retention was 40%. The individual AA utilization for whole-body protein retention indicates that arginine, histidine, methionine, glutamic acid, and glycine are the absorbed AA most efficiently used for meat production.

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