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## Amino acid utilization of lactating dairy cows when diets are changed from an alfalfa-based diet to cereal straw-based diets



## B. Wang, H.Z. Sun, N.N. Xu, K.J. Zhu, J.X. Liu\*

Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou 310058, PR China

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#### ABSTRACT

This experiment assessed the utilization of amino acids (AA) by the mammary gland of cows when diets are changed from an alfalfa hay-based diet (AH) to cereal straw-based diets using corn stover (CS) or rice straw (RS). Thirty multiparous Holstein dairy cows were selected and randomly assigned to 1 of 3 treatments (n = 10). After 13 weeks' feeding, arterial and venous plasma were collected every 6 h over 2 days and AA concentrations measured. The cows fed the CS or RS diet had lower milk and protein yield despite similar dry matter intake. The digestive AA flow was predicted to be lower in the cows fed CS or RS than in AH-fed cows. The arterial concentration of methionine and valine was lower in the cows fed RS than in cows fed AH. The mammary uptake of most essential AA, especially branched-chain AA, methionine, and arginine, was greater in the AH-fed cows than in cows fed CS or RS. The ratio of mammary uptake to milk output of methionine was lower in the RS-fed cows than that in the cows fed AH, with a value below 1 for both. In summary, the insufficient supply of free AA from arterial plasma presented to the mammary gland and lower mammary plasma flow restrict the mammary AA uptake for milk protein synthesis when energy is limited. On the other hand, the utilization of leucine and methionine may be a limiting factor for milk protein synthesis and lactation performance when corn stover or rice straw is fed to dairy cows.

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## 1. Introduction

Cereal straws such as corn stover and rice straw are widely available, but their use is limited because of reduced milk production when these forages are fed to dairy cows (Pang et al., 2008; Sarnklong et al., 2010). In our previous study (Wang et al., 2014), cows fed cereal straw-based diets had lower milk yield, lower milk protein yield and lower gross utilization

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Abbreviations: AA, mino acids; AD, facid detergent fiber; AH, total mixed ration containing alfalfa hay as the main forage; AV, arterio-venous; BCAA, branched-chain amino acids; CS, total mixed ration containing corn stover as the main forage; CP, crude protein; DM, dry matter; EAA, essential amino acids; MD, the ratio of milk AA output to estimated digestive AA flow; MG, mammary gland; MP, metabolizable protein; MPF, mammary plasma flow; N, nitrogen; NDF, neutral detergent fiber; NEAA, non-essential amino acids; RS, total mixed ration containing rice straw as the main forage; UOAA, uptake to output ratios across the mammary gland.

<sup>\*</sup> Corresponding author at: Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Scienc, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, P. R. China.

E-mail address: liujx@zju.edu (J.X. Liu).

Ingredients and nutrient composition (mean  $\pm$  SD) of the total mixed ration containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage (n = 3).

	Treatment						
Items	AH	CS	RS				
Dietary ingredient, g/kg of DM							
Ground corn grain	270	270	270				
Wheat bran	51.0	51.0	51.0				
Soybean meal	127	127	127				
Cottonseed meal	43.0	43.0	43.0				
Beet pulp	10.0	0.0	0.0				
Corn silage	150	150	150				
Alfalfa hay	230	0.0	0.0				
Chinese wild grass hay	70.0	0.0	0.0				
Corn stover	0.0	300	0.0				
Rice straw	0.0	0.0	300				
Urea	0.0	10.0	10.0				
Premix <sup>a</sup>	49.0	49.0	49.0				
Nutrient composition <sup>b</sup> , g/kg of DM							
Organic matter	$923\pm29.6$	$923\pm20.6$	$901 \pm 19.9$				
Crude protein	$168\pm1.51$	$163\pm3.98$	$162\pm5.81$				
Neutral detergent fiber	$318\pm14.3$	$367 \pm 10.8$	$365 \pm 12.8$				
Acid detergent fiber	$175\pm1.50$	$209 \pm 1.45$	$202\pm1.49$				
Non-fiber carbohydrate	$408\pm40.0$	$353\pm22.6$	$348 \pm 44.6$				
Ca	$8.10\pm0.63$	$6.21\pm0.71$	$5.60\pm0.67$				
Р	$4.63\pm0.18$	$4.72\pm0.14$	$\textbf{3.89} \pm \textbf{0.40}$				
NE <sub>L,</sub> MJ/kg	6.57	6.07	5.98				

<sup>a</sup> Formulated to provide (per kilogram of DM): 174g of zeolite powder, 1.25g of yeast, 25g of mold adsorbent (Solis Mos, Novus International Inc., St. Charles, MO), 21.44g of KCl, 41.25g of MgO, 150g of Salt, 187.5g of NaHCO<sub>3</sub>, 84g of Ca, 15g of P, 125,000 IU of vitamin A, 750,000 IU of vitamin D3, 937.5 IU of vitamin E, 1750 mg of Zn, 17.5 mg of Se, 28.75 mg of I, 375 mg of Fe, 15 mg of Co, 556.5 mg of Mn and 343.75 mg of Cu.

<sup>b</sup> Non-fiber carbohydrate = 100-% Neutral detergent fiber – % crude protein – % ether extract – % ash; NE<sub>L</sub> = Net energy for lactating, calculated based on the Ministry of Agriculture of P. R. China recommendations (MoA, 2004).

efficiency of dietary nitrogen (N) than did those fed alfalfa hay, mainly due to reduced supply of metabolizable protein (MP) and net energy of lactation in a corn stover or rice straw-based diet compared to an alfalfa hay-based diet. Lower milk protein synthesis in the cows fed cereal straws in place of alfalfa hay (Wang et al., 2014) could be attributed to the lower energy consumption and lower microbial synthesis as well as the ingestion of dietary amino acids (AA).

Improved efficiency of N utilization may be achieved by improving the efficiency of AA utilization and by manipulating dairy diets to optimize mammary gland (MG) uptake of essential AA (EAA; Arriola Apelo et al., 2014). An improved representation of the biology of milk protein synthesis should include individual EAA rather than MP and the effects of multiple nutrients on AA utilization (Arriola Apelo et al., 2014). In our previous study (Wang et al., 2014), it was not clear how AA were metabolized and utilized by dairy cows when alfalfa hay was replaced with cereal straws. Therefore, it was necessary to understand AA metabolism in the MG under these diverse nutritional conditions.

The objectives of this study were to investigate the effects of replacing an alfalfa/Chinese wild rye hay mixture with corn stover or rice straw made isonitrogenous by the addition of urea on AA uptake by the MG of dairy cows in mid-lactation, and to identify the potential limiting factors for milk production from AA aspect under these dietary conditions.

## 2. Materials and methods

## 2.1. Animals and experimental design

The experimental procedures were approved by the Animal Care Committee at Zhejiang University (Hangzhou, China) and were in accordance with the university's guidelines for animal research.

Feeding and management of the experimental cows have been described previously (Wang et al., 2014). Thirty multiparous Holstein dairy cows (body weight =  $600 \pm 52.0$  kg, milk yield =  $30.0 \pm 3.53$  kg/d, day in milk =  $160 \pm 27.8$  d, parity =  $3.4 \pm 1.57$ ; mean  $\pm$  SD) were blocked into 10 groups according to day in milk and milk yield, and were allocated to 3 treatments randomly within groups. Three isonitrogenous treatment diets contained a similar concentrate mixture (550 g/kg) and 150 g/kg corn silage, with 300 g/kg of the diet being treatment forages [dry matter (DM) basis, Table 1]: (1) 230 g/kg alfalfa hay and 70 g/kg Chinese wild rye hay (AH); (2) 300 g/kg corn stover (CS); and (3) 300 g/kg rice straw (RS). Diets were made isonitrogenous by the addition of 10 g/kg urea to the cereal straw-based diets. The chemical composition of individual forage has been described previously (Wang et al., 2014). Cows were fed and milked 3 times daily at 06.30, 14.00, and 20.00 h. After 13 weeks on treatment as part of the larger study, lactation performance was monitored and samples of feeds, milk, and arterial and venous plasma were collected.

## 2.2. Sampling, measurements, and analyses

During the 14th week of the feeding trial, the amount of the feed offered was recorded every day and was adjusted to allow for 5–10% orts; DM intake was calculated based on the feed offered and orts. Milk production was recorded for the first 3 consecutive days, and milk samples were collected on the third day using milk-sampling devices (Waikato Milking Systems NZ Ltd., Waikato, Hamilton, New Zealand). One 50-ml aliquot of the milk sample was collected at each milking of the sampling day, proportional to the yield (4:3:3, composite), and the milk was pooled on individual basis. One 50-ml aliquot of the composited milk sample, with added bronopol tablets (milk preservative, D & F Control Systems, San Ramon, CA), was stored at 4 °C for future analysis of protein, fat, lactose, milk urea N, total solid, and somatic cell by infrared analysis (Laporte and Paquin, 1999) with a spectrophotometer (Foss-4000; Foss Electric A/S, Hillerod, Denmark). Another 50-mL aliquot of milk was stored at -20 °C for later analysis of AA composition.

Feeds were sampled every other day during the collection period, and a total of 3 feed samples were collected. The airdried samples were ground through a 2-mm screen (Wiley Laboratory Mill; Arthur H. Thomas Co., Philadelphia, PA) and then through a 1 mm screen in a Cyclotec mill (Tecator 1093; Tecator AB, Hoganas, Sweden) before analysis of DM (105 °C for 5 h), CP (method 988.05; AOAC, 1990), acid detergent fiber (method 973.18; AOAC, 1990), and neutral detergent fiber (Van Soest et al., 1991), without sodium sulfite and amylase added. The nutrient compositions of the 3 diets are listed in Table 1.

The AA in feed and milk was analyzed with norleucine as an internal standard by an Automatic AA Analyzer (Hitachi High-technologies Corporation, Tokyo, Japan). Before analysis, the samples of feed and milk were pretreated to hydrolyze the protein according to the modified protocol (Delgado-Elorduy et al., 2002). Briefly, samples were hydrolyzed in HCl at 112 °C for 24 h prior to analysis, at a 1:1 (v/w) ratio of HCl to sample, with simultaneous replenishment with highly pure N<sub>2</sub> and pre-oxidized with performic acid to protect the sulphur groups. The HCl solution (6 M) contained sodium sulfite (1 g/L) and phenol (1.1 mg/ml). The hydrolysate was purged continuously with N<sub>2</sub> to remove the acid at a temperature of 60 °C. After hydrolysis, the samples were first filtered through 0.45  $\mu$ m and then through 0.22  $\mu$ m nylon syringe filter units (Fisher Scientific, Pittsburgh, PA) placed in microcentrifuge tubes (catalog no. 05-664-34, Fisher Scientific). The AA concentrations of hydrolyzed samples were corrected for losses during hydrolysis by recovery factors that were determined by regressing the hydrolysis time and the concentration following hydrolysis of a composite of feed samples or of skim milk at 6 h intervals from 6 to 48 h (Delgado-Elorduy et al., 2002).

Blood samples from the coccygeal artery (arterial sample) and superficial epigastric vein (mammary venous sample) were collected from all of the cows over 2 consecutive days. The blood samples were taken every 6 h as described below: at 06.00, 12.00, 18.00 and 24.00 h on the first sampling day; and at 09.00, 15.00, 21.00, and 03.00 h on the second day. The cows were standing for at least 10 min prior to blood sampling to minimize fluctuations and approximate steady-state conditions for blood flow.

Blood samples were collected using lithium-heparin-containing vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) and then centrifuged at 3000g for 15 min at 4 °C, pipetted, and stored at -80 °C until analysis. Plasma samples were pooled per cow on an equal volume basis after thawing and analyzed by duplicate by each pooled sample. Pooled plasma was pretreated according to the modified method as described before (Mackle et al., 1999). In brief, ice-cold sulfosalicylic acid (50 g/L) was added to the plasma (1:4, v/v) to precipitate the protein. The sample was then centrifuged at 8320g for 30 min at 4 °C. The supernatant was then filtered through 0.45  $\mu$ m and then through 0.22  $\mu$ m nylon syringe filter units (Fisher Scientific, Pittsburgh, PA) and placed in microcentrifuge tubes (catalog no. 05-664-34, Fisher Scientific) before being analyzing with norleucine as an internal standard in an Automatic AA Analyzer (Hitachi High-technologies Corporation, Tokyo, Japan).

Digestive AA flow was estimated by the NRC model (NRC, 2001;Pacheco et al., 2012). All information regarding feed amounts, milk production, age, days pregnant, days in milk, and body weight for each cow was entered into the NRC model. The nutrient composition for individual feed ingredients published elsewhere (Wang et al., 2014) was also entered. The body condition score was assumed to be 3.3 for the cows at a late lactation stage in the current study. The ratio of milk AA output to estimated digestive AA flow (M:D) was calculated.

The MPF was estimated by Fick's principle, which assumes the stoichiometric transfer of free phenylalanine + tyrosine from plasma into milk protein, using phenylalanine and tyrosine as internal markers (Mepham, 1982), with an allowance for a 35 g/kg contribution from blood-borne proteins (Cant et al., 1993) and with the exception that free milk phenylalanine and tyrosine values are neglected. The concentration of phenylalanine + tyrosine in milk true protein was used to make the calculation. Other indices reflecting AA utilization by the MG included arterial concentration, arterio-venous (AV) differences, clearance rate, mammary uptake, and the ratio of mammary AA uptake to milk AA output (U:O).

### 2.3. Calculations and statistical analysis

The values reflecting AA utilization by the MG were calculated as below:

M:D (%) = AA output in milk (g/d)/predicted digestive AA flow  $(g/d) \times 100$ .

MPF (L/d) = (milk phenylalanine + tyrosine)  $(g/d) \times 0.965/[AV difference of (phenylalanine + tyrosine) (g/L)].$ 

Arterial free AA supply (g/d) = Arterial free AA concentration  $(g/L) \times MPF(L/d)$ .

Clearance rate of AA in the MG was calculated from the model of Hanigan et al. (1998). Positive values indicate uptake, and negative values indicate output by the MG.

Dietary amino acid composition in dairy cows fed the different treatment TMR containing alfalfa hay (AH), corn stover (CS), and rice straw (RS) as the main forage.

	Diet, g/kg DM					Milk, g/kg				
Items*	AH	CS	RS	SEM	P-value	AH	CS	RS	SEM	P-value
EAA	47.1 <sup>a</sup>	40.7 <sup>b</sup>	38.8 <sup>b</sup>	1.01	<0.01	16.0	15.5	14.6	1.15	0.71
Arginine	6.67	6.31	6.04	0.23	0.16	1.14	1.08	1.02	0.09	0.66
Histidine	3.02 <sup>a</sup>	2.67 <sup>b</sup>	2.64 <sup>b</sup>	0.09	0.03	1.29	1.12	1.08	0.14	0.58
Isoleucine	4.67 <sup>a</sup>	3.81 <sup>b</sup>	3.73 <sup>b</sup>	0.21	0.02	1.57	1.49	1.42	0.11	0.83
Leucine	9.10 <sup>a</sup>	8.29 <sup>b</sup>	7.89 <sup>b</sup>	0.25	0.04	3.28	3.27	3.06	0.23	0.74
Lysine	5.51 <sup>a</sup>	4.45 <sup>b</sup>	4.24 <sup>b</sup>	0.27	0.03	2.86	2.85	2.68	0.19	0.75
Methionine	1.51	1.34	1.30	0.07	0.33	0.88	0.88	0.82	0.06	0.73
Phenylalanine	5.71 <sup>a</sup>	5.18 <sup>b</sup>	4.67 <sup>b</sup>	0.13	<0.01	1.61	1.54	1.45	0.11	0.63
Threonine	4.81 <sup>a</sup>	3.78 <sup>b</sup>	3.78 <sup>b</sup>	0.15	<0.01	1.42	1.39	1.31	0.11	0.72
Valine	5.89 <sup>a</sup>	4.75 <sup>b</sup>	4.64 <sup>b</sup>	0.15	<0.01	1.96	1.86	1.78	0.14	0.70
NEAA	60.8 <sup>a</sup>	51.8 <sup>b</sup>	50.1 <sup>b</sup>	1.48	<0.01	18.5	18.3	17.3	1.18	0.74
Alanine	7.11 <sup>a</sup>	5.89 <sup>b</sup>	5.91 <sup>b</sup>	0.28	0.03	1.14	1.12	1.05	0.08	0.67
Aspartate	12.1 <sup>a</sup>	9.29 <sup>b</sup>	9.02 <sup>b</sup>	0.36	<0.01	2.64	2.55	2.42	0.19	0.73
Cysteine	1.62	1.61	1.54	0.05	0.62	-	-	-	-	-
Glutamate	19.2 <sup>a</sup>	18.2 <sup>ab</sup>	17.2 <sup>b</sup>	0.40	0.04	7.35	7.63	7.13	0.45	0.72
Glycine	5.21 <sup>a</sup>	4.20 <sup>b</sup>	4.19 <sup>b</sup>	0.14	<0.01	0.65	0.63	0.6	0.04	0.69
Proline	6.91 <sup>a</sup>	5.23 <sup>b</sup>	5.02 <sup>b</sup>	0.25	<0.01	3.25	3.09	2.96	0.23	0.68
Serine	5.89 <sup>a</sup>	4.75 <sup>b</sup>	4.71 <sup>b</sup>	0.27	0.03	1.91	1.88	1.79	0.11	0.64
Tyrosine	2.78 <sup>a</sup>	2.67 <sup>ab</sup>	2.49 <sup>b</sup>	0.06	0.02	1.56	1.44	1.37	0.11	0.48
BCAA	19.7 <sup>a</sup>	16.8 <sup>b</sup>	16.1 <sup>b</sup>	0.53	<0.01	6.81	6.62	6.26	0.45	0.74
Total AA	108 <sup>a</sup>	92.4 <sup>b</sup>	88.9 <sup>b</sup>	2.48	<0.01	34.5	33.8	31.9	2.31	0.72

<sup>a</sup> EAA = Essential amino acids; NEAA = Non-essential amino acids; BCAA = Branched-chain amino acids (Valine, Isoleucine, and Leucine). <sup>a</sup> Means within the same row with different superscripts differ (*P*<0.05).

<sup>b</sup>Means within the same row with different superscripts differ (P<0.05).

Clearance rate  $(L/h) = MPF(L/h) \times AV$  difference of AAi (g/L)/Venous concentration of AAi (g/L).

Mammary uptake (g/d) = AV difference  $(g/L) \times MPF(L/d)$ .

U:O = AA uptake in MG (g/d)/AA output in milk (g/d).

Total AA was the sum of EAA and NEAA. The EAA included arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine; and the NEAA included alanine, aspartate, cysteine, glutamate, glycine, proline, serine, and tyrosine; and the BCAA included isoleucine, leucine, and valine. Group 1 AA included methionine, phenylalanine, tyrosine, and histidine; and Group 2 AA included arginine, valine, isoleucine, leucine, lysine, and threonine.

The variance of the data for all measurements was analyzed as a completely randomized design using PROC MIXED of SAS (Institute, 2000). A completely randomized block design was used, with diet as the fixed effect and cows within each diet as a random effect. The statistical model was as follows:

$$Yij = \mu + Bi + Tj + Eij$$

where Yij is the dependent variable,  $\mu$  is the overall mean, Bi is the random effect of block I, Tj is the diet effect, and Eij is the error term. The covariance structure with the least Akaike information criterion [ar (1)] was used. The results were reported as least squares means. The Bonferroni test was used to compare the means between treatments. The statistical significance of main effects was declared at  $P \le 0.05$ .

## 3. Results

#### 3.1. Dietary amino acids composition, feed intake, and milk production performance

The AA profiles of the 3 diets and respective milk are listed in Table 2. The dietary contents of most AA were significantly greater in diet AH compared with diet CS or RS on a DM basis. The milk AA profiles were similar among the 3 treatments. The results of feed intake and milk production are listed in Table 3. There was no difference among the 3 treatments for DM intake (P=0.15), milk fat yield (P=0.26), percentage of milk fat (P=0.28), milk protein (P=0.37), milk lactose (P=0.06) and total solid (P=0.53), as well as for somatic cell (P=0.15). Yields of milk (P<0.01), milk protein (P<0.05), lactose (P<0.01), and total solid (P<0.05) as well as feed efficiency (P<0.01) were higher for the cows fed AH than those fed RS or CS, with no difference between the cows fed RS and CS. Milk urea N was higher (P<0.01) for the cows fed RS than cows fed AH or CS, with no difference between the cows fed AH and CS.

#### 3.2. Estimated digestive flow of essential amino acids

The estimated digestive EAA flow and the calculated M:D are listed in Table 4. The estimated digestive flow of isoleucine (P < 0.05), lysine (P < 0.01), methionine (P < 0.01), phenylalanine (P < 0.01), threonine (P < 0.01), valine (P < 0.01),

Lactation performance during the sampling week in dairy cows fed the total mixed ration containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage.

	Treatment				
Items*	AH	CS	RS	SEM	P-value
DM intake, kg/d	17.6	16.9	17.0	0.24	0.15
Yield, kg/d					
Milk	24.1 <sup>a</sup>	18.4 <sup>b</sup>	19.8 <sup>b</sup>	0.72	< 0.01
Fat	0.92	0.78	0.86	0.053	0.26
Protein	0.78 <sup>a</sup>	0.62 <sup>b</sup>	0.63 <sup>b</sup>	0.033	0.01
Lactose	1.20 <sup>a</sup>	0.90 <sup>b</sup>	0.94 <sup>b</sup>	0.042	< 0.01
Total solid	3.07 <sup>a</sup>	2.43 <sup>b</sup>	2.58 <sup>b</sup>	0.119	0.01
Milk composition, g/kg					
Fat	37.9	42.4	42.8	2.08	0.28
Protein	32.2	34.1	31.7	1.18	0.37
Lactose	49.4	48.9	47.2	0.59	0.06
Total solid	127	133	130	3.31	0.53
MUN, mg/dl	14.3 <sup>b</sup>	15.8 <sup>b</sup>	17.6 <sup>a</sup>	0.42	< 0.01
Feed efficiency	1.38 <sup>a</sup>	1.09 <sup>b</sup>	1.17 <sup>b</sup>	0.048	< 0.01
N conversion, g/kg	267 <sup>a</sup>	228 <sup>b</sup>	223 <sup>b</sup>	11.7	0.05
Somatic cell, $\times 10^3$	629	421	216	134.2	0.15

<sup>a-c</sup> Means within a row that have different superscripts differ significantly (P<0.05).

\*MUN = Milk urea nitrogen; Feed efficiency = Milk yield (kg/d)/DM intake (kg/d); N conversion = Milk protein yield (g/d)/Crude protein intake (g/d) × 1000.

#### Table 4

Estimated digestive essential amino acids flow and ratios of milk output: digestive flow (M:D) of AA in dairy cows fed the different treatment TMR containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage.

	Treatment				
Items*	AH	CS	RS	SEM	P-value
Digestive EAA flow, g/d					
Arginine	84.4 <sup>a</sup>	78.6 <sup>b</sup>	79.9 <sup>ab</sup>	1.73	0.04
Histidine	37.1	35.4	37.2	0.64	0.09
Isoleucine	81.5 <sup>a</sup>	75.7 <sup>b</sup>	75.1 <sup>b</sup>	1.26	0.01
Leucine	139 <sup>a</sup>	126 <sup>b</sup>	131 <sup>ab</sup>	2.23	< 0.01
Lysine	114 <sup>a</sup>	102 <sup>b</sup>	101 <sup>b</sup>	1.72	< 0.01
Methionine	29.0 <sup>a</sup>	27.1 <sup>b</sup>	26.7 <sup>b</sup>	0.45	< 0.01
Phenylalanine	83.9 <sup>a</sup>	77.1 <sup>b</sup>	78.0 <sup>b</sup>	1.28	< 0.01
Threonine	81.4 <sup>a</sup>	74.1 <sup>b</sup>	69.7 <sup>b</sup>	1.25	< 0.01
Valine	92.4 <sup>a</sup>	84.7 <sup>b</sup>	84.9 <sup>b</sup>	1.44	< 0.01
BCAA	313 <sup>a</sup>	286 <sup>b</sup>	291 <sup>b</sup>	4.92	< 0.01
EAA	743 <sup>a</sup>	681 <sup>b</sup>	682 <sup>b</sup>	11.67	< 0.01
M:D, %					
Arginine	28.6	25.4	25.4	7.20	0.41
Histidine	64.3	58.6	57.2	7.20	0.78
Isoleucine	40.4	36.4	37.6	2.71	0.61
Leucine	52.0	47.8	46.6	2.95	0.45
Lysine	56.4	51.4	53.2	3.39	0.61
Methionine	70.2	59.9	61.7	4.13	0.23
Phenylalanine	42.5	36.7	37.1	2.29	0.20
Threonine	38.6	34.5	37.4	2.27	0.45
Valine	45.8	40.4	41.8	2.76	0.41
BCAA	47.2	42.6	42.9	2.80	0.49
EAA	44.8	39.5	40.4	2.55	0.35

<sup>a-b</sup> Means within the same row with different superscripts differ (P < 0.05).

<sup>\*</sup>The digestive AA flow was estimated by NRC (2001) model; M:D (%) = Mammary AA output in milk (g/d)/Digestive AA flow (g/d) × 100. EAA = Essential AA; BCAA = Branched-chain AA (Valine, Isoleucine, and Leucine).

BCAA (P<0.01), and EAA (P<0.01) were greater in cows fed AH than in cows fed CS or RS, with no difference between the CS and RS diets. The digestive flow of arginine (P<0.05) and leucine (P<0.01) was greater in cows fed AH than the CS-fed cows (P<0.01), with no differences between CS or AH and RS. No difference was found in M:D for all the estimated EAA among the 3 diets (P>0.05).

#### 3.3. Arterial and venous plasma free amino acids

The arterial concentrations Met (P<0.01) and value (P=0.04) in AH-fed cows were higher than in cows fed RS, with no difference between the cows fed CS and those fed AH or RS (Table 5). The cows fed RS had higher arterial concentration of

Concentration of free amino acid in arterial and venous plasma in dairy cows fed the total mixed ration containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage.

	Arterial plasma, mg/L					Venous p	olasma, mg/L			
Items*	AH	CS	RS	SEM	P-value	AH	CS	RS	SEM	P-value
EAA	147	133	135	5.26	0.16	108 <sup>a</sup>	102 <sup>a</sup>	88.7 <sup>b</sup>	3.04	< 0.01
Arginine	10.2	9.61	11.4	0.53	0.07	4.44	5.51	4.50	0.44	0.18
Histidine	11.1	10.6	11.3	0.82	0.83	9.41	9.40	9.80	0.72	0.93
Isoleucine	20.0	16.1	16.9	1.32	0.11	16.4 <sup>a</sup>	12.7 <sup>b</sup>	11.8 <sup>b</sup>	0.87	< 0.01
Leucine	22.2	18.2	18.3	1.21	0.05	13.1 <sup>a</sup>	11.7 <sup>a</sup>	8.30 <sup>b</sup>	0.80	< 0.01
Lysine	15.4	14.0	15.1	0.80	0.43	9.30	9.02	7.71	0.56	0.14
Methionine	6.33 <sup>a</sup>	5.73 <sup>ab</sup>	5.02 <sup>b</sup>	0.26	< 0.01	4.90	4.54	4.14	0.31	0.22
Phenylalanine	7.91	7.60	7.54	0.32	0.73	5.00	5.21	4.12	0.38	0.13
Threonine	12.9	13.7	13.6	0.43	0.43	10.7	12.2	11.1	0.48	0.10
Valine	41.2 <sup>a</sup>	37.4 <sup>ab</sup>	35.3 <sup>b</sup>	1.50	0.04	35.3ª	31.7 <sup>ab</sup>	27.3 <sup>b</sup>	1.33	< 0.01
NEAA	111	112	118	2.74	0.14	94.3	95.5	99.0	2.38	0.37
Alanine	13.3	13.5	13.9	0.59	0.78	11.3	11.8	11.8	0.56	0.73
Aspartate	5.02	4.71	5.64	1.02	0.83	4.35	3.26	3.72	0.42	0.21
Cysteine	23.0 <sup>b</sup>	25.4 <sup>a</sup>	22.0 <sup>b</sup>	0.64	< 0.01	24.7	25.0	24.5	0.51	0.78
Glutamate	30.5	30.9	33.0	1.09	0.24	21.1	23.4	23.2	1.21	0.35
Glycine	17.8 <sup>b</sup>	16.6 <sup>b</sup>	21.6 <sup>a</sup>	0.89	< 0.01	16.1 <sup>b</sup>	15.6 <sup>b</sup>	19.6 <sup>a</sup>	0.82	< 0.01
Proline	4.62	2.23	5.44	1.00	0.12	3.12	1.53	3.31	0.67	0.16
Serine	9.32	9.51	9.90	0.57	0.73	7.42	7.93	8.13	0.31	0.24
Tyrosine	9.01	9.11	9.32	0.36	0.75	7.61	7.13	7.22	0.34	0.54
BCAA	83.4 <sup>a</sup>	71.7 <sup>ab</sup>	70.5 <sup>b</sup>	3.83	0.05	64.8 <sup>a</sup>	56.1 <sup>ab</sup>	47.4 <sup>b</sup>	2.68	< 0.01
Total AA	258	245	253	7.15	0.46	203	198	188	4.07	0.05

 $^{a-b}$  Means within the same row with different superscripts differ (P<0.05).

\*EAA = Essential amino acids; NEAA = Non-essential amino acids; BCAA = Branched-chain amino acids (Valine, Isoleucine, and Leucine).



**Fig. 1.** Mammary plasma flow (MPF) in the dairy cows fed the total mixed ration containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage, which was estimated by the Fick principle based on the uptake and output of phenylalanine and tyrosine; MPF=(milk phenylalanine+tyrosine)  $\times$  0.965/[Arterio-venous difference of (phenylalanine+tyrosine)]. The a-b or A-B above the column indicates that the values within same color column with different superscripts differ (*P*<0.05). The bars indicate the standard error.

glycine (P < 0.01) than those fed CS or AH, and cows fed CS had higher arterial concentration of cysteine (P < 0.01) than those fed RS or AH.

Venous concentrations of EAA (P<0.01) and leucine (P<0.01) were higher in AH or CS-fed cows than in cows fed RS, with no difference between the cows fed CS or AH (Table 5). Concentration of isoleucine (P<0.01) was I higher in AH-fed cows than those fed RS and CS. The value and total BCAA were greater in cows fed AH than cows fed RS (P<0.01), with no difference between the cows fed CS and those fed AH or RS. For cows fed RS, venous glycine concentration was greater (P<0.01) than in cows fed CS or AH.

#### 3.4. Utilization of amino acids by the mammary gland

## 3.4.1. Mammary plasma flow

The diets had significant effect on the estimated MPF rates in terms of either liter per day or per kilogram of milk (Fig. 1). The cows fed AH (750 L/kg milk) had higher (P < 0.05) MPF values than cows fed RS (512 L/kg milk). The MPF of CS-fed cows (687 L/kg milk) were between the two, with no difference between the cows fed AH and RS.

The arterial-venous difference (A-V difference) and arterial supply of amino acid in dairy cows fed the total mixed ration containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage.

	A-V difference, mg/L				Arterial s	Arterial supply <sup>1</sup> , g/d				
Items*	AH	CS	RS	SEM	P-value	AH	CS	RS	SEM	P-value
EAA	38.4	31.3	45.8	4.14	0.07	2648 <sup>a</sup>	1651 <sup>b</sup>	1339 <sup>b</sup>	184.3	< 0.01
Arginine	5.83 <sup>ab</sup>	4.05 <sup>b</sup>	6.89 <sup>a</sup>	0.58	< 0.01	186 <sup>a</sup>	120 <sup>b</sup>	111 <sup>b</sup>	12.4	< 0.01
Histidine	1.23	1.56	1.52	0.26	0.47	188 <sup>a</sup>	137 <sup>b</sup>	109 <sup>b</sup>	11.7	< 0.01
Isoleucine	3.71	3.44	5.06	1.04	0.47	361 <sup>a</sup>	197 <sup>b</sup>	168 <sup>b</sup>	30.8	< 0.01
Leucine	9.12	6.64	10.0	1.12	0.14	403 <sup>a</sup>	223 <sup>b</sup>	181 <sup>b</sup>	30.8	< 0.01
Lysine	6.17	5.04	7.41	1.00	0.25	277 <sup>a</sup>	175 <sup>b</sup>	148 <sup>b</sup>	20.3	< 0.01
Methionine	1.38	1.16	0.93	0.18	0.16	114 <sup>a</sup>	73.2 <sup>b</sup>	52.4 <sup>b</sup>	9.0	< 0.01
Phenylalanine	2.89	2.43	3.43	0.36	0.17	143 <sup>a</sup>	93.2 <sup>b</sup>	74.4 <sup>b</sup>	10.0	< 0.01
Threonine	2.17	1.42	2.41	0.39	0.20	234 <sup>a</sup>	168 <sup>ab</sup>	138 <sup>b</sup>	17.8	< 0.01
Valine	5.89	5.67	8.05	0.94	0.17	743 <sup>a</sup>	464 <sup>b</sup>	358 <sup>b</sup>	54.0	< 0.01
NEAA	16.6	16.1	18.7	3.62	0.86	1995 <sup>a</sup>	1394 <sup>b</sup>	1188 <sup>b</sup>	136.1	< 0.01
Alanine	2.04	1.70	2.13	0.33	0.73	239 <sup>a</sup>	168 <sup>b</sup>	155 <sup>b</sup>	17.6	< 0.01
Aspartate	0.73	1.45	1.89	1.19	0.77	92.2	61.1	54.3	15.8	0.21
Cysteine	-1.71	0.36	-2.49	0.87	0.08	417 <sup>a</sup>	316 <sup>ab</sup>	222 <sup>b</sup>	30.6	< 0.01
Glutamate	9.39	7.47	9.80	1.46	0.50	545 <sup>a</sup>	388 <sup>b</sup>	332 <sup>b</sup>	37.5	< 0.01
Glycine	1.72	1.14	1.89	0.29	0.11	321 <sup>a</sup>	206 <sup>b</sup>	214 <sup>b</sup>	23.4	< 0.01
Proline	1.52	0.84	2.18	1.28	0.76	75.1	31.4	53.4	12.5	0.09
Serine	1.89	1.63	1.81	0.64	0.92	166 <sup>a</sup>	116 <sup>b</sup>	96 <sup>b</sup>	11.0	< 0.01
Tyrosine	1.43	2.01	2.18	0.27	0.11	162 <sup>a</sup>	113 <sup>b</sup>	92 <sup>b</sup>	11.3	< 0.01
BCAA	18.6	15.6	23.2	2.82	0.19	1507 <sup>a</sup>	884 <sup>b</sup>	707 <sup>b</sup>	114.1	< 0.01
Total AA	54.9	47.4	64.5	6.95	0.25	4643 <sup>a</sup>	3046 <sup>b</sup>	2526 <sup>b</sup>	313.2	< 0.01

<sup>a-b</sup> Means within the same row with different superscripts differ (P < 0.05).

\*EAA = Essential amino acids; NEAA = Non-essential amino acids; BCAA = Branched-chain amino acids (Valine, Isoleucine, and Leucine).

 $^{1}$ Arterial supply of amino acid (g/d) = Arterial amino acid concentration (g/L) × Mammary plasma flow (L/d).

## 3.4.2. Arterial-venous difference and arterial free amino acids supply

The AV difference of arginine showed greater in cows fed RS than cows fed CS (P<0.01), with no difference between the AH-fed cows and cows fed RS and CS (Table 6). No difference was found for other AA among the 3 diets (P>0.05). The supply of all the analyzed arterial plasma free AA, except for aspartate and proline, to MG was greater in cows fed AH than cows fed CS or RS (P<0.05; Table 6).

## 3.4.3. Clearance rate of amino acids

The clearance rate of AA was listed in Table 7. The mammary clearance of total EAA (P < 0.01), arginine (P < 0.05), phenylalanine (P < 0.05), threonine (P < 0.01), and glycine (P < 0.05) was lower in the CS-fed cows than cows fed AH, with no difference between RS-fed cows and cows fed AH or CS. Compared to the AH-fed cows, the cows fed RS had lower clearance rate of methionine (P < 0.05), glutamate (P < 0.05), and serine (P < 0.01). The mammary clearance of total AA across the MG was faster (P < 0.01) for cows fed AH than cows fed CS or RS, with no difference between the cows fed CS and RS.

## 3.4.4. Mammary uptake of amino acids

The cows fed the AH diet had a significant higher mammary uptake of EAA (P<0.01; Table 7), including arginine (P<0.01), isoleucine (P<0.05), leucine (P<0.05), lysine (P<0.05), methionine (P<0.01), phenylalanine (P<0.01), and threonine (P<0.01); of NEAA, including glutamine (P<0.01), glycine (P<0.05), serine (P<0.05); of BCAA (P<0.05), and of total AA (P<0.01) from the plasma across their MG than cows fed the CS or RS diets, with no difference between the cows fed CS and RS.

## 3.4.5. Uptake to output ratios for amino acids across the mammary gland

The U:O across the MG for EAA (P<0.01) and group 2 AA (P<0.01) were greater in the cows fed AH than cows fed CS or RS, with no difference between the cows fed CS and RS (Table 8). The U:O of methionine in cows fed RS were the lowest among all the EAA; and it was lower (P<0.05) than that in AH-fed cows and below 1. The U:O of leucine has a tendency to be greater in cows fed AH than cows fed CS or RS.

## 4. Discussion

In ruminants, AA intake is a meaningless way for expressing the real supply of AA to the animals in comparison with monogastric animals. Feed undegraded, microbial, and endogenous protein flow to and digestion in the small intestine must be incorporated into models to predict the AA supply (Lapierre et al., 2006). It is sufficiently accurate to use NRC model for prediction of duodenal EAA flows (Pacheco et al., 2012). The lower digestive AA flow in the cows fed CS and RS than in AH-fed cows in the current study was consistent with the total MP flow reported by Wang et al. (2014) with the same

Clearance rate and uptake of amino acid by the mammary gland of dairy cows fed the different treatment TMR containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage.

	Clearance rate <sup>1</sup> , L/h				Uptake <sup>2</sup> , g/d					
Items*	AH	CS	RS	SEM	P-value	AH	CS	RS	SEM	P-value
EAA	340 <sup>a</sup>	181 <sup>b</sup>	228 <sup>ab</sup>	31.5	< 0.01	827 <sup>a</sup>	425 <sup>b</sup>	463 <sup>b</sup>	77.7	< 0.01
Arginine	1112 <sup>a</sup>	414 <sup>b</sup>	713 <sup>ab</sup>	169.3	0.03	105 <sup>a</sup>	52.2 <sup>b</sup>	66.1 <sup>b</sup>	8.6	< 0.01
Histidine	282	250	270	49.6	0.90	48.3	43.2	42.1	7.1	0.81
Isoleucine	478	256	233	83.6	0.10	139 <sup>a</sup>	63.2 <sup>ab</sup>	54.5 <sup>b</sup>	21.5	0.02
Leucine	575	293	543	114.3	0.19	170 <sup>a</sup>	78.9 <sup>b</sup>	93.3 <sup>ab</sup>	23.1	0.03
Lysine	488	303	430	81.6	0.28	107 <sup>a</sup>	61.1 <sup>b</sup>	70.2 <sup>ab</sup>	11.7	0.03
Methionine	299 <sup>a</sup>	153 <sup>ab</sup>	77.0 <sup>b</sup>	48.0	0.01	30.4 <sup>a</sup>	14.3 <sup>b</sup>	7.57 <sup>b</sup>	3.3	< 0.01
Phenylalanine	439 <sup>a</sup>	244 <sup>b</sup>	356 <sup>ab</sup>	51.5	0.05	48.6 <sup>a</sup>	29.0 <sup>b</sup>	32.5 <sup>b</sup>	3.4	< 0.01
Threonine	145 <sup>a</sup>	51.0 <sup>b</sup>	86 <sup>ab</sup>	18.5	< 0.01	37.1 <sup>a</sup>	14.5 <sup>b</sup>	21.3 <sup>b</sup>	4.3	< 0.01
Valine	176	92.0	116	27.9	0.12	141	69.5	74.3	21.8	0.06
NEAA	163	107	96.7	19.3	0.06	353 <sup>a</sup>	231 <sup>ab</sup>	205 <sup>b</sup>	37.7	0.03
Alanine	230	125	178	38.0	0.17	55.2	32.0	38.5	7.2	0.09
Aspartate	305	337	509	288.4	0.87	12.8	20.8	13.4	17.1	0.94
Cysteine	-46.7	2.96	-50.5	17.2	0.07	-29.9	0.78	-30.2	10.6	0.09
Glutamate	354 <sup>a</sup>	202 <sup>ab</sup>	176 <sup>b</sup>	41.0	0.01	169 <sup>a</sup>	98.9 <sup>b</sup>	89.9 <sup>b</sup>	16.1	< 0.01
Glycine	134 <sup>a</sup>	47.1 <sup>b</sup>	106 <sup>ab</sup>	21.8	0.03	48.8 <sup>a</sup>	16.7 <sup>b</sup>	38.1 <sup>ab</sup>	7.8	0.03
Proline	519	532	470	312.4	0.99	26.2	11.9	17.3	14.4	0.80
Serine	342 <sup>a</sup>	217 <sup>ab</sup>	137 <sup>b</sup>	34.9	< 0.01	50.9 <sup>a</sup>	32.6 <sup>b</sup>	22.6 <sup>b</sup>	4.6	< 0.01
Tyrosine	140	143	133	20.1	0.94	24.7	23.5	21.5	3.0	0.74
BCAA	322	164	204	48.8	0.08	450 <sup>a</sup>	211 <sup>b</sup>	223 <sup>ab</sup>	63.8	0.03
Total AA	255 <sup>a</sup>	144 <sup>b</sup>	159 <sup>b</sup>	20.1	< 0.01	1180 <sup>a</sup>	656 <sup>b</sup>	667 <sup>b</sup>	94.1	< 0.01

<sup>a-c</sup> Means within the same row with different superscripts differ (P < 0.05).

\*EAA = Essential amino acids; NEAA = Non-essential amino acids; BCAA = Branched-chain amino acids (Valine, Isoleucine, and Leucine).

<sup>1</sup> Clearance rate (L/h) = Mammary plasma flow (L/h) × Arterio-venous difference of AAi (g/L)/Venous concentration of AAi (g/L).

<sup>2</sup> Uptake (g/d) = Arterio-venous difference  $(g/L) \times$  Mammary plasma flow (L/d).

#### Table 8

Amino acid uptake (g/d) to output (g/d) ratios (U:O) across the mammary gland (amino acid uptake in mammary/amino acid output in milk) in dairy cows fed the total mixed ration containing alfalfa hay (AH), corn stover (CS), or rice straw (RS) as the main forage.

	Treatment				
Items*	AH	CS	RS	SEM	P-value
EAA	2.09 <sup>a</sup>	1.52 <sup>b</sup>	1.62 <sup>b</sup>	0.118	< 0.01
Arginine	3.92	2.67	3.31	0.440	0.11
Histidine	1.79	2.38	2.15	0.421	0.60
Isoleucine	3.43ª	2.36 <sup>ab</sup>	1.99 <sup>b</sup>	0.369	0.04
Leucine	2.01	1.34	1.58	0.174	0.08
Lysine	1.58	1.18	1.36	0.188	0.42
Methionine	1.39 <sup>a</sup>	0.90 <sup>ab</sup>	0.48 <sup>b</sup>	0.159	< 0.01
Phenylalanine	1.28	1.03	1.13	0.065	0.07
Threonine	1.11	0.61	0.92	0.171	0.12
Valine	2.74	2.08	2.13	0.310	0.22
NEAA	0.78	0.69	0.62	0.111	0.62
Alanine	1.92	1.56	1.80	0.258	0.56
Aspartate	0.23	0.44	0.25	0.310	0.87
Cysteine	-	-	-	_	-
Glutamate	0.95	0.70	0.64	0.135	0.23
Glycine	2.93	1.47	3.46	0.593	0.11
Proline	0.36	0.21	0.31	0.162	0.90
Serine	1.11	0.96	0.67	0.138	0.10
Tyrosine	0.64	0.90	0.80	0.068	0.07
BCAA	2.55	1.77	1.83	0.198	0.05
Group 1	1.24	1.22	1.12	0.082	0.54
Group 2	2.28 <sup>a</sup>	1.57 <sup>b</sup>	1.73 <sup>b</sup>	0.141	< 0.01

 $^{\rm a-b}$  Means within the same row with different superscripts differ (P<0.05).

\*EAA = Essential amino acids; NEAA = Non-essential amino acids; BCAA = Branched-chain amino acids (Valine, Isoleucine, and Leucine); Group 1 (Methionine, Phenylalanine, Tyrosine, and Histidine); Group 2 (Arginine, Valine, Isoleucine, Leucine, Lysine, and Threonine).

dietary treatments, indicating that the most essential AA might be in shortage and limited milk protein synthesis even at similar dietary CP content.

The supply of all the arterial plasma free EAA to the MG was greater in cows fed AH than cows fed CS or RS, similar to their digestive flow, suggesting that the arterial plasma AA supply is dependent on the amount of digestive EAA flowing to the duodenum (Apelo et al., 2014; Arriola Apelo et al., 2014). The supply of AA to the MG is the product of arterial AA

concentration in blood (plasma) and MPF. The MPF was also reported as a linear function relative to milk yield (Hanigan et al., 2002), consistent with the results of milk yield in this study (R<sup>2</sup> = 0.36). Published data confirmed that the infusion of EAA reduced mammary blood flow (Doepel and Lapierre, 2010), but infusion of NEAA and total AA did not (Metcalf et al., 1996). The MPF in the cows fed AH could not explain this because of the higher MPF value in the cows fed AH than in the RS-fed cows under similar arterial plasma EAA concentrations even if methionine and valine showed significant difference. Additionally, starch supplementation by abomasal infusion resulted in an increase in MPF via increased energy supply (Rius et al., 2010). The supply of glucose to Holstein cows may increase MPF, affecting the net removal of EAA (Rulquin et al., 2004). In our study, the lower MPF might be probably accounted for the lower intake of net energy for lactation in the cows fed CS and RS compared to AH-fed cows.

Increased mammary clearance rates of AA are part of a local adaptive mechanism to support milk protein synthesis, wherein clearance rate is matched to cellular needs (Rius et al., 2010), consistent with the greater value of clearance rate of AA that support for higher milk protein yield in cows fed AH. Compared to arterial concentrations and mammary uptake, the U:O may be more useful for identifying the limiting AA (Bequette et al., 1998; Mabjeesh et al., 2005; Lapierre et al., 2012). The U:O of EAA from pooled samples using an AA analyzer showed greater range value compared with individual samples by isotopic dilution in the review of Lapierre et al. (2012), with range from 0.88 to 4.18 for arginine and from 0.59 to 1.18 for methionine. Whereas, this review did not include the forage source such as corn stover or rice staw. Therefore, the U:O of individual AA in our study that seems a little greater or lower compared with the published data in lactating dairy cow may be supplementary for the database of U:O.

The U:O of methionine was lower than 1.00. In this case, the output in the milk is greater than the estimated uptake, suggesting that some contribution of peptides containing methionine would have been possible, although free AA in the blood are considered to be the major precursors of milk protein (Bequette et al., 1998; Hanigan et al., 2002). Mabjeesh et al. (2005) reported that peptides could contribute to the AA used for milk protein synthesis by MG, with an estimated value of 7–18%. There is also evidence that blood free AA may be insufficient to meet tissue needs (Vanhatalo et al., 1999). The results of in vivo studies using a dual-labeled tracer technique indicate that the peptide-binding EAA can be used for milk protein synthesis (Backwell et al., 1996; Bequette et al., 1999). Histidine, leucine, lysine, methionine, phenylalanine, tyrosine, and valine can also be used in peptide form to synthesize casein (Bequette et al., 1999; Tagari et al., 2008).

Our observation was consistent with previous studies that most EAA and several NEAA were used for milk protein synthesis as precursor or generating for other AA or catabolised due to the transamination or deamination (Lapierre et al., 2012; Arriola Apelo et al., 2014). The glutamete is considered to be a N donor for other AA including methionine (Ivanisevic et al., 2015). The U:O ratio for glutamete did not change among treatments, but was lower than 1.00, suggesting its possible shortsge under our dietary formulation. The negative values or below one of U:O also indicate the anabolism of these AA, which may mainly attribute to the transamination roles occurred in MG (Lapierre et al., 2012; Ivanisevic et al., 2015). The U:O of leucine in the cows fed CS or RS tended to be lower than that in the AH-fed cows, indicating that MG extensively and more easily degrades the greater leucine from cows fed CS or RS in order to synthesize glutamate, alanine, and aspartate during lactation (Li et al., 2009). Further oxidation of leucine would also supply energy to the MG (Thivierge et al., 2002).

During lactation, BCAA catabolism in the MG is markedly increased (DeSantiago et al., 1998) due to the enhanced expression of BCAA transferase (Van Hall et al., 1996). Lei et al. (2012) reported that reductions in insulin and growth hormone along with increases in cortisol and glucagon with lactation act in concert to stimulate BCAA catabolism for glutamate and glutamine syntheses. Thus, BCAA are taken up well in excess of their output in milk protein and contribute to the pool of NEAA required for milk protein synthesis on an AH diet (Mepham, 1982). Published results have demonstrated that the BCAA play a role as metabolic signals and interact with energy to transfer messages about nutrition status for milk protein synthesis through the mammalian target of rapamycin pathway (Burgos et al., 2010). The effect of BCAA on the stimulation of protein synthesis through the mammalian target of rapamycin pathway can preferentially affect the expression of specific genes such as S6K1 and 4E-BP1 (Burgos et al., 2010). In accordance with published results (Korhonen et al., 2002), the uptake of NEAA, with the exception of alanine, glutamate, and glycine in the AH-fed cows, was inconsistent and often less than their output in milk. The results suggest that as part of their nutritional function, such AA might serve not only as substrates for protein synthesis but also as signaling molecules that regulate the process (Arriola Apelo et al., 2014). Further research is needed to clarify this.

## 5. Conclusion

The quality of forage did markedly affect arterial supply of EAA, MPF, and mammary EAA uptake. The lower U:O of methionine in the RS-fed cows and the lower U:O of leucine in the CS- and RS-fed cows than in the AH-fed cows indicate that shortage of methionine and leucine may restrict milk protein synthesis. The results from this study provide evidence of the importance of an adequate supply of methionine and leucine when corn stover or rice straw is included in the diets for lactating dairy cows.

## **Conflict of interest**

The authors declare that there is no conflict of interest.

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## References

OAC (Association of Official Analytical Chemists).1990. Official methods of analysis. 15th ed. AOAC, Arlington, VA.

- Apelo, S.A., Bell, A.L., Estes, K., Ropelewski, J., de Veth, M.J., Hanigan, M.D., 2014. Effects of reduced dietary protein and supplemental rumen-protected essential amino acids on the nitrogen efficiency of dairy cows. J. Dairy Sci. 97, 5688–5699.
- Arriola Apelo, S.I., Knapp, J.R., Hanigan, M.D., 2014. Invited review: current representation and future trends of predicting amino acid utilization in the lactating dairy cow. J. Dairy Sci. 97, 4000–4017.
- Backwell, F.R.C., Bequette, B.J., Wilson, D., Matcalf, J.A., Franklin, M.F., Beever, D.E., Lobley, G.E., MacRae, J.C., 1996. Evidence for utilization of peptides for milk protein synthesis in the lactating dairy goat in vivo. Am. J. Physiol. 271, R955–R960.
- Bequette, B.J., Backwell, F.R.C., Crompton, L.A., 1998. Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. J. Dairy Sci. 81, 2540–2559.
- Bequette, B.J., Backwell, F.R.C., Kyle, C.E., Calder, A.G., Buchan, V., Crompton, L.A., France, J., MacRae, J.C., 1999. Vascular sources of phenylalanine, tyrosine, lysine, and methionine for casein synthesis in lactating goats. J. Dairy Sci. 82, 362–377.
- Burgos, S.A., Dai, M., Cant, J.P., 2010. Nutrient availability and lactogenic hormones regulate mammary protein synthesis through the mammalian target of rapamycin signaling pathway. J. Dairy Sci. 93, 153–161.
- Cant, J.P., DePeters, E.J., Baldwin, R.L., 1993. Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. J. Dairy Sci. 76, 762–764.
- DeSantiago, S., Torres, N., Suryawan, A., Tovar, A.R., Hutson, S.M., 1998. Regulation of branched-chain amino acid metabolism in the lactating rat. J. Nutr. 128, 1165–1171.
- Delgado-Elorduy, A., Theurer, C.B., Huber, J.T., Alio, A., Lozano, O., Sadik, M., Cuneo, P., DeYoung, D., Simas, J., Webb Jr., K.E., Tagari, H., 2002. Splanchnic and mammary nitrogen metabolism by dairy cows fed dry-rolled or steam-flaked sorghum grain. J. Dairy Sci. 85, 148–159.
- Doepel, L., Lapierre, H., 2010. Changes in production and mammary metabolism of dairy cows in response to essential and nonessential amino acid infusions. J. Dairy Sci. 93, 3264–3274.
- Hanigan, M.D., France, J., Wray-Cahen, D., Beever, D.E., Lobley, G.E., Reutzel, L., Smith, N.E., 1998. Alternative models for analyses of liver and mammary transorgan metabolite extraction data. Brit. J. Nutr. 79, 63–78.
- Hanigan, M.D., Crompton, L.A., Bequette, B.J., Mills, J.A.N., France, J., 2002. Modelling mammary metabolism in the dairy cow to predict milk constituent yield, with emphasis on amino acid metabolism and milk protein production: model evaluation. J. Theor. Biol. 217, 311–330.
- SAS Institute, 2000. SAS User's Guide. Statistics, Version 8.01. SAS Inst., Inc., Cary, NC. Ivanisevic, J., Elias, D., Deguchi, H., Averell, P.M., Kurczy, M., Johnson, C.H., Tautenhahn, R., Zhu, Z., Watrous, J., Jain, M., Griffin, J., Patti, G.J., Siuzdak, G., 2015. Arteriovenous blood metabolomics: a readout of intra-Tissue metabostasis. Sci. Rep. 5, 12757.
- Korhonen, M., Vanhatalo, A., Huhtanen, P., 2002. Evaluation of isoleucine, leucine, and valine as a second-limiting amino acid for milk production in dairy cows fed grass silage diet. J. Dairy Sci. 85, 1533–1545.
- Lapierre, H., Pacheco, D., Berthiaume, R., Ouellet, D.R., Schwab, C.G., Dubreuil, P., Holtrop, G., Lobley, G.E., 2006. What is the true supply of amino acids for a dairy cow? J. Dairy Sci. 89, E1–E14 (E. Suppl.).
- Lapierre, H., Lobley, G.E., Doepel, L., Raggio, G., Rulquin, H., Lemosquet, S., 2012. Triennial lactation symposium: mammary metabolism of amino acids in dairy cows. J. Dairy Sci. 90, 1708–1721.

Laporte, M.F., Paquin, P., 1999. Near-infrared analysis of fat, protein, and casein in cow's milk. J. Agric. Food Chem. 47, 2600–2605.

- Lei, J., Feng, D.Y., Zhang, Y.L., Dahanayaka, S., Li, X.L., Yao, K., Wang, J.J., Wu, Z.L., Dai, Z.L., Wu, G.Y., 2012. Regulation of leucine catabolism by metabolic fuels in mammary epithelial cells. Amino Acids 43, 2179–2189.
- Li, P., Knabe, D.A., Kim, S.W., Lynch, C.J., Hutson, S.M., Wu, G.Y., 2009. Lactating porcine mammary tissue catabolizes branched-chain amino acids for glutamine and aspartate synthesis. J. Nutr. 139, 1502–1509.
- Mabjeesh, S.J., Gal-Garber, O., Milgram, J., Feuermann, Y., Cohen-Zinder, M., Shamay, A., 2005. Aminopeptidase N gene expression and abundance in caprine mammary gland is influenced by circulating plasma peptide. J. Dairy Sci. 88, 2055–2064.
- Mackle, T.R., Dwyer, D.A., Bauman, D.E., 1999. Effects of branched-chain amino acids and sodium caseinate on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82, 161–171.

Mepham, T.B., 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65, 287–298.

- Metcalf, J.A., Crompton, L.A., Wray-Cahen, D., Lomax, M.A., Sutton, J.D., Beever, D.E., MacRae, J.C., Bequette, B.J., Backwell, F.R.C., Lobley, G.E., 1996. Responses in milk constituents to intravascular administration of two mixtures of amino acids to dairy cows. J. Dairy Sci. 79, 1425–1429.
- MoA (Ministry of Agriculture of P.R. China). 2004. Feeding Standard of Dairy Cattle (NY/T 34–2004). MOA, Beijing, China.
- Nutrient requirements of dairy cattle, 2001. 7th rev. edn. Natl. Acad. Sci., Washington, DC.
- Pacheco, D., Patton, R.A., Parys, C., Lapierre, H., 2012. Ability of commercially available dairy ration programs to predict duodenal flows of protein and essential amino acids in dairy cows. J. Dairy Sci. 95, 937–963.
- Pang, Y.Z., Liu, Y.P., Li, X.J., Wang, K.S., Yuan, H.R., 2008. Improving biodegradability and biogas production of corn stover through sodium hydroxide solid state pretreatment. Energy Fuels. 22, 2761–2766.
- Rius, A.G., Appuhamy, J.A.D.R.N., Cyriac, J., Kirovski, D., Becvar, O., Escobar, J., McGilliard, M.L., Bequette, B.J., Akers, R.M., Hanigan, M.D., 2010. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. J. Dairy Sci. 93, 3114–3127.
- Rulquin, H., Rigout, S., Lemosquet, S., Bach, A., 2004. Infusion of glucose directs circulating amino acids to the mammary gland in well-fed dairy cows. J. Dairy Sci. 87, 340–349.
- Sarnklong, C., Cone, J.W., Pellikaan, W., Hendriks, W.H., 2010. Utilization of rice straw and different treatments to improve its feed value for ruminants: a review. Asian. Aust. J. Anim. Sci 23, 680–692.
- Tagari, H., Webb Jr, K., Theurer, B., Huber, T., DeYoung, D., Cuneo, P., Santos, J.E.P., Simas, J., Sadik, M., Alio, A., Lozano, O., Delgado-Elorduy, A., Nussio, L., Bittar, C.M.M., Santos, F., 2008. Mammary uptake, portal-drained visceral flux, and hepatic metabolism of free and peptide-bound amino acids in cows fed steam-flaked or dry-rolled sorghum grain diets. J. Dairy Sci. 91, 679–697.
- Thivierge, M.C., Petitclerc, D., Bernier, J.F., Couture, Y., Lapierre, H., 2002. Variations in mammary protein metabolism during the natural filling of the udder with milk over a 12-h period between two milkings: leucine kinetics. J. Dairy Sci. 85, 2974–2985.
- Van Hall, G., MacLean, D.A., Saltin, B., Wagenmakers, A.J.M., 1996. Mechanisms of activation of muscle branched-chain a-keto acid dehydrogenase during exercise in man. J. Physiol. 494, 899–905.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Vanhatalo, A., Huhtanen, P., Toivonen, V., Varvikko, T., 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combination with methionine and lysine. J. Dairy Sci. 82, 2674–2685.
Wang, B., Mao, S.Y., Yang, H.J., Wu, Y.M., Wang, J.K., Li, S.L., Shen, Z.M., Liu, J.X., 2014. Effects of alfalfa and cereal straw as a forage source on nutrient digestibility and lactation performance in lactating dairy cows. J. Dairy Sci. 97, 7706–7715.