Asynchronous Supply of Indispensable Amino Acids Reduces Protein Deposition in Milk-Fed Calves


Abstract
A balanced supply of indispensable amino acids (AA) is required for efficient protein synthesis. Different absorption kinetics (e.g., free vs. protein-bound AA) may, however, create asynchrony in postabsorptive availability of individual AA, thereby reducing the efficiency of protein deposition. We studied the effects of AA asynchrony on protein metabolism in growing, milk-fed calves. In 2 experiments, each with a change-over design including 8 calves, a milk replacer deficient in Lys and Thr was used. In Expt. 1, L-Lys and L-Thr were parenterally supplemented, either in synchrony (SYN), asynchrony (ASYN), or partial asynchrony (PART) with dietary AA. In Expt. 2, L-Lys and L-Thr were orally supplemented, either in SYN or ASYN with dietary AA. In Expt. 1, digested protein was used less efficiently for growth for ASYN (31.0%) than for SYN (37.7%), with PART being intermediate (36.0%). Indicator AA oxidation tended (P = 0.06) to be higher for ASYN. In Expt. 2, the efficiency of protein utilization was lower for ASYN (34.9%) than for SYN (46.6%). Calves spared AA from oxidation when the limiting AA were provided in excess after a short period (<24 h) of deprivation. Restoring AA balance by parenteral supplementation resulted in a 19% lower efficiency of digestible protein utilization than by oral supplementation, likely caused by splanchnic oxidation of imbalanced AA in excess to Thr. In conclusion, asynchronous availability of individual indispensable AA reduces the efficiency by which digested protein is retained in milk-fed calves. Furthermore, an AA imbalance in the splanchnic tissues may result in disproportionate AA oxidation.

Introduction
Efficient conversion of dietary protein into body protein requires a balanced supply of indispensable amino acids (AA), sufficient amounts of dispensable AA, and simultaneous availability of sufficient energy (1). The quality of dietary protein can be estimated from the AA composition and from digestibility coefficients of individual AA. This led to the protein digestibility-corrected AA score for humans (2) and the ideal protein concept for pigs (3) and poultry (4). Such concepts for digestibility-corrected AA score for humans (2) and the ideal coefficients of individual AA. This led to the protein estimated from the AA composition and from digestibility sufficent energy (1). The quality of dietary protein can be amounts of dispensable AA, and simultaneous availability of an AA imbalance in the splanchnic tissues may result in disproportionate AA oxidation. J. Nutr. 142: 2075–2082, 2012.

Diurnal fluctuations in postabsorptive AA availability occur as a consequence of either the meal pattern or the kinetics of protein digestion. Slow rates of protein digestion stimulate postprandial protein retention in humans (3,6), but the influence of meal pattern on protein metabolism is not conclusive from human and rat studies (7). The latter is probably due to the large variation in protein sources tested and the relatively low growth rates in those studies. In fast-growing, milk-fed calves, an increase in the number of meals resulted in greater protein deposition compared with single-meal patterns (8). Similarly, growing pigs deposited protein-bound AA more efficiently than crystalline AA when fed once daily, but not when fed 6 meals/d (10,11). Crystalline AA are indeed absorbed shortly after feeding, whereas protein-bound AA need to be hydrolyzed prior to absorption, which causes a delay in absorption (12,13). This would explain the interaction between meal frequency and protein source and suggests that kinetics in postabsorptive AA availability may affect postprandial protein utilization. At a low meal frequency, diurnal variation in kinetics of individual, indispensable AA may lead to an imbalanced AA pattern at the site of deposition, which could limit the amount of protein deposited compared with synchronous availability of AA. So far, the effects of synchronizing the availability of AA mutually on protein metabolism
have hardly been studied in mammals. Early studies on AA asynchrony in rats (14–18) showed inconclusive results, which are possibly due to the large variation in protein sources (zein, wheat gluten, fish protein, crystalline AA mixtures) and indispensable AA used (Trp, Lys, Met+Cys, Thr). The relatively low growth rates in those studies may also have contributed to the variable results. In rapidly growing animals, such as milk-fed calves, effects of a suboptimal timing of AA supply on N balance will likely be more pronounced. To date, vegetable proteins have replaced substantial amounts of dairy proteins in milk replacer for calves, with subsequent addition of crystalline AA to reach ideal AA patterns (19,20); hence, asynchronous absorption kinetics of indispensable AA may occur.

Therefore, the objective of this study was to quantify potential effects of asynchronous availability of AA on protein metabolism and energy partitioning between protein and lipid deposition in milk-fed calves. In addition, diurnal changes in whole body AA oxidation were measured for explaining putative effects on N balance.

### Materials and Methods

Two studies were designed for assessing the effects of AA asynchrony on protein and energy metabolism in heavy, milk-fed calves. A milk replacer deficient in Lys and Thr was used in both studies. In the first study, calves received parenteral supplementation of i-Lys plus i-Thr either in synchrony (SYN), asynchrony (ASYN), or partial asynchrony (PART) with AA from the diet. In the second study, i-Lys and i-Thr were orally supplemented either in SYN or ASYN with dietary AA. Both studies were used in 4 trials with 2 calves of similar age and体重 (BW) at start. A trial consisted of 3 experimental periods of 14 d each. In each period, calves were assigned to 1 of 3 treatments of parenteral AA supplementation and all calves were subjected to each treatment once. The order of the treatments was randomized, in total, this resulted in 24 observations (n = 8/treatment). The supplemented AA (i.e., i-Lys and i-Thr, see below) were i.v. infused into the jugular vein from either 0 to 2 (PART), 2 to 4 (SYN), or 8 to 10 h (ASYN) after each of the 2 daily feedings. Based on portal AA fluxes in calves fed vegetable protein-based milk replacer (21), these time intervals were expected to create PART, SYN, or ASYN in availability of protein-bound AA and supplemented AA, respectively. Measurements were conducted during the last 7 d of each period.

In Expt. 2, 8 male Holstein Friesian calves were used in 4 trials with 2 calves of similar age and 150 ± 2.6 kg body weight (BW) at start. A trial consisted of 3 experimental periods of 14 d each. In each period, calves were assigned to 1 of 3 treatments of parenteral AA supplementation and all calves were subjected to each treatment once. The order of the treatments was randomized, in total, this resulted in 24 observations (n = 8/treatment). The supplemented AA (i.e., i-Lys and i-Thr, see below) were i.v. infused into the jugular vein from either 0 to 2 (PART), 2 to 4 (SYN), or 8 to 10 h (ASYN) after each of the 2 daily feedings. Based on portal AA fluxes in calves fed vegetable protein-based milk replacer (21), these time intervals were expected to create PART, SYN, or ASYN in availability of protein-bound AA and supplemented AA, respectively. Measurements were conducted during the last 7 d of each period.

Calves were prepared with venous catheters (Becton Dickinson) in each jugular vein for the purpose of parenteral AA supplementation (Expt. 1), blood sampling, and stable isotope infusion (Expt. 1 and 2).

### Diets and feeding

Milk replacer contained whey and soluble wheat as protein sources (Table 1). Lys (5.6 g/kg) and Thr (6.7 g/kg) were the most limiting indispensable AA, due to the high contribution (80%) of wheat to the dietary protein. Calves were fed at 2.25× the metabolizable energy (ME) requirements for maintenance. Milk replacer was reconstituted with water (140 g/L) and supplied at a temperature of ~40°C in a bucket. Roughage was not supplied and calves were allowed 15 min to consume the milk.

### Table 1 Ingredient and nutrient composition of the milk replacer for Expt. 1 and 2

<table>
<thead>
<tr>
<th>Ingredient, g/kg</th>
<th>Nutrient, g/kg dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey</td>
<td>130 DM, g/kg</td>
</tr>
<tr>
<td>Soluble wheat protein</td>
<td>200 Crude ash</td>
</tr>
<tr>
<td>Fat-filled whey</td>
<td>400 Crude protein</td>
</tr>
<tr>
<td>Lactose</td>
<td>237 Crude fat</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>13.8 Lactose</td>
</tr>
<tr>
<td>Mono potassium phosphate</td>
<td>15.6 Gross energy, MJ/kg</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>1.80 Lys</td>
</tr>
<tr>
<td>Iron sulfate</td>
<td>0.13 Met</td>
</tr>
<tr>
<td>Vitamin and mineral premix</td>
<td>2.0 Cys</td>
</tr>
<tr>
<td></td>
<td>4.5 Lys</td>
</tr>
<tr>
<td></td>
<td>2.1 Trp</td>
</tr>
<tr>
<td></td>
<td>8.7 Ille</td>
</tr>
</tbody>
</table>

1 Analyzed content, unless indicated otherwise.
2 Contained 50% fat: palm oil (26.5%), coconut oil (20%), rapeseed oil (2.5%), and emulsifier E471 11%.
3 Provided per kilogram of the experimental diet: calcium, 7.5 g; phosphorus, 6.2 g; magnesium, 1.5 g; retinol, 7.5 mg; cholecalciferol, 50 μg; α-tocopherol, 80 mg; ascorbic acid, 130 mg; zinc, 86 mg; copper, 5 mg; iron, 51 mg; manganese, 13 mg; selenium, 0.1 mg; cobalt, 1.0 mg.
4 Calculated values.

In Expt. 1, calves were fed twice daily (at 0600 and 1800 h) and supplemental i-Lys and i-Thr were i.v. infused. Pyrogen-free i-Lys hydrochloride and i-Thr (BUFA B.V.) was dissolved in saline (18.6 and 13.0 g/L, respectively) and the pH was adjusted to 7.4 by adding 1 mol/L sodium dihydrogen phosphate. This solution was filtered over a 0.22-μm filter (Millipore) and autoclaved for 60 min at 120°C. Osmolarity in the infusate was ~350 mOsm/kg. The infusate was administered via the jugular vein to the calves using a peristaltic pump (Watson-Marlow) at a rate adjusted to the feed allowance. The supplemented Lys and Thr contributed for 6.0 g/kg to total Lys (11.6 g/kg) and for 6.2 g/kg to total Thr (12.9 g/kg) supply.

In Expt. 2, all calves were fed 4 equal-sized meals (at 0000, 0600, 1200, and 1800 h) per day. Supplemental i-Lys (6.0 g/kg) and i-Thr (6.2 g/kg) was now provided in either 1 (1200 h; ASYN) or all meals (SYN). Total daily supplementation with AA was identical for both treatments.

### Housing and measurements

In both experiments, calves were individually housed in 1 of 2 identical climatic respiration chambers (2.5 × 1.5 × 2.0 m). Within each chamber, calves were housed in metabolic cages (1.85 × 0.75 m). Animals in the 2 separate chambers could see each other. Temperature was maintained at 18°C, relative humidity at 65%, and air velocity was ~0.2 m/s. Calves were exposed to 13 h of light (50 lx; from 0530 to 1830 h) and 11 h of partial darkness (6 lx). In Expt. 2, lights were also switched on from 0000 to 0030 h, because calves were fed at 0000 h.

Harnesses for the fecal collection bags were attached 5 d before the start of each experiment. A 7-d balance trial was performed for measuring apparent total tract digestibility and deposition of protein and fat. All procedures for sample collection and analyses were similar to those previously described (8). Briefly, feed, feed refusals, feces, urine, and aerial NH₃ were quantitatively collected. The exchange of oxygen, carbon dioxide, and methane was measured in 6-min intervals during 7 d (22).

### Isotope administration

In Expt. 1, on d 2 of the balance trial, 7.6 mmol of L-[1-13C]Leu (99.4 atom%; Sigma-Aldrich) was added to the 1800 h meal as an indicator for oxidation of imbalanced AA (23). Blood samples of 5 mL were taken in 30-min intervals from ~30 to 600 min relative to feeding for measuring 13C enrichment of plasma leucine (24). On d 6, [1-13C]sodium bicarbonate (99.0 atom%; Sigma-Aldrich) was i.v. infused for 2 h after the 1800 h meal along with the supplemented AA at a rate of 2.4 mmol/h for measuring bicarbonate sequestration.
In Expt. 2, [15N15N]urea (99.1 atom% ; Sigma-Aldrich) was dissolved in sterile saline as 50 mmol/L and was infused in the left jugular vein at a rate of 0.24 mmol/h with a calibrated syringe pump (Infors AG) for studying diurnal kinetics of urea production. The continuous infusion (55 h) started on d 1 at 1700 h and was preceded by a priming dose to enrich the body urea pool to ~0.50 atom percent excess. On d 3, blood samples of 5 mL were taken from the right jugular vein during 24 h at 30-min intervals. Blood was immediately transferred into lithium heparin tubes (Becton Dickinson) and stored on ice until plasma was collected after centrifugation at 1500 x g for 10 min. Plasma samples were stored at −20°C pending analyses. On d 4, 5.7 mmol of [1-13C]leucine (Sigma-Aldrich) was supplied as an indicator AA for oxidation, with the feed at 0000 h (i.e., no supplementation of Lys and Thr at 0000 h), and the 13C label was corrected for the 14N-15N enrichment of urea, plasma samples were deproteinized by addition of trichloroacetic acid (8%, w/v), plasma was allowed to react with ninhydrin and absorption of the formed complex was measured at 570 nm in a spectrophotometer (Beckman Coulter DU-530). Plasma leucine was analyzed with an enzymatic kit (Liquicolor, Human). For measuring 15N enrichment of urea, plasma samples were deproteinized by adding 5 mL cold methanol and stored at −20°C for 1 h. Then, the samples were centrifuged at 1500 x g for 20 min at 4°C and the supernatant was passed over a cation exchange column (Dowex 50WX2–200, Sigma-Aldrich) for separating urea from other N-containing components in plasma. The column was washed with distilled water until the pH was neutral. The eluent was evaporated using a rotary evaporator. One milliliter of distilled water was added to the residue and this solution was transferred into a 1.5-mL microcentrifuge tube. The solution was freeze-dried and 100 μL of distilled water was added, mixed, and transferred into a tin capsule. After evaporation at 40°C, the capsules were analyzed for 15N enrichment by isotope ratio mass spectrometry (Finnigan MAT). Nitrogen isotopomers for urea ([15N]N, [15N14N], [15N214N], and [15N214N]N) were analyzed in pooled urine samples per calf as described by Lobley et al. (25).

For plasma KIC enrichment, samples were derivatized as described by Matthews et al. (26) and 13C enrichment was measured by GC-MS (model 9571A, Hewlett-Packard) with selected ion monitoring. Enrichment of 13C in expired CO2 was measured in 6-min intervals by nondispersive infrared spectrometry (ABB) as previously described (27).

Calculations. For each balance period, digestible energy intake was derived from intake of feed energy (gross energy) minus fecal energy loss. Intake of ME was calculated as the difference between digestible energy intake and the sum of energy losses in urine and methane. From the gaseous exchangers, heat production was calculated according to the formula of Bouwerr (28). Energy retention was calculated by subtracting heat production from ME intake. Retention of N was calculated from N in feed and N in excreta. Energy retained as protein was derived from retained N assuming 23.6 kJ/kg of protein. Energy retention as fat was calculated by subtracting energy retained as protein from total energy retention. The respiratory quotient (RQ) was calculated as CO2 production divided by O2 consumption. Balance period means were calculated for all variables and hourly means were calculated for heat production and RQ.

Table 2. Effects of time of parenteral L-Lys plus L-Thr supplementation on general performance and N utilization for growth in milk-fed calves (Expt. 1) 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SYN</th>
<th>PART</th>
<th>ASYN</th>
<th>SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>175</td>
<td>166</td>
<td>160</td>
<td>8.3</td>
<td>0.535</td>
</tr>
<tr>
<td>Weight gain, g/d</td>
<td>1200</td>
<td>1200</td>
<td>1220</td>
<td>173.6</td>
<td>0.836</td>
</tr>
<tr>
<td>Dry matter intake, g/d</td>
<td>51.6</td>
<td>51.5</td>
<td>51.4</td>
<td>0.42</td>
<td>0.159</td>
</tr>
<tr>
<td>N intake, mg/(kg0.75·d)</td>
<td>1786</td>
<td>1788</td>
<td>1792</td>
<td>38.5</td>
<td>0.823</td>
</tr>
<tr>
<td>Fecal N excretion, mg/(kg0.75·d)</td>
<td>170</td>
<td>147</td>
<td>158</td>
<td>15.2</td>
<td>0.368</td>
</tr>
<tr>
<td>Digestible N intake, mg/(kg0.75·d)</td>
<td>1616</td>
<td>1641</td>
<td>1634</td>
<td>16.4</td>
<td>0.897</td>
</tr>
<tr>
<td>Urinary N excretion, mg/(kg0.75·d)</td>
<td>968</td>
<td>1016</td>
<td>1099</td>
<td>15.1</td>
<td>0.013</td>
</tr>
<tr>
<td>Nitrogen retention, mg/(kg0.75·d)</td>
<td>609</td>
<td>589</td>
<td>505</td>
<td>17.2</td>
<td>0.095</td>
</tr>
<tr>
<td>Efficiency of N utilization for growth, %</td>
<td>34.2</td>
<td>33.0</td>
<td>28.3</td>
<td>0.070</td>
<td></td>
</tr>
<tr>
<td>Of N intake</td>
<td>37.7</td>
<td>36.0</td>
<td>31.0</td>
<td>0.94</td>
<td>0.046</td>
</tr>
</tbody>
</table>

1 Data are least-squares means, n = 5. Means within a row with a common letter differ, P < 0.05 (pairwise comparison). ASYN, asynchrony; BW, body weight; PART, partial asynchrony; SYN, synchrony.

2 Calves were supplied with a milk replacer deficient in Lys and Thr at 0600 and 1800 h, and L-Lys plus L-Thr was supplemented via i.v. infusion at 2–4 (SYN), 0–2 (PART), or 8–10 h (ASYN) after each feeding.

Results

General performance and nutrient digestibility (Expt. 1). Three calves (9 observations) were excluded from the statistical analysis. One calf developed phlebitis during the first experimental period and the other 2 calves had feed refusals (>10% of feed allowance). The BW of calves at the start of the experiment averaged 167 ± 8.3 kg and daily BW gain was similar (1231 ± 174 g) for all treatments (Table 2). The apparent total tract digestibility of dry matter (94.9%), protein (91.1%), energy (95.0%), crude fat (95.8%), lactose (99.9%), and ash (78.4%) were similar for Expt. 2. Sequestration of bicarbonate was measured in all calves in Expt. 1 by assessing the recovery of [13C]bicarbonate as [13CO2] from the 2-h bicarbonate infusion during a 10-h period. This recovery did not differ between treatments, averaging 92.7 ± 0.7%.

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and digestible nutrient intakes (data not shown) did not differ between treatments.

**Protein and energy metabolism (Expt. 1).** Urinary N excretion was greater \( (P < 0.05) \) for ASYN than for PART and SYN (Table 2). Nitrogen retention tended to decrease \( (P = 0.10) \) with decreasing synchrony in AA supply. The efficiency of digestible N utilization for protein deposition was 18% lower \( (P < 0.05) \) for ASYN than for SYN, with PART being intermediate. Plasma \( \alpha \)-amino N responded to milk intake and peaked at 2–4 h after feeding (Supplemental Fig. 1). Although this response did not differ between treatments, plasma \( \alpha \)-amino N increased numerically during the 2-h periods of AA infusion for ASYN and PART, whereas this did not occur for SYN. Heat production and fat deposition were not affected by treatment (Supplemental Table 1). Within-day patterns of heat production and RQ were not affected by treatment. Whole-body Leu oxidation tended to be higher \( (P = 0.06) \) for ASYN than for SYN and PART as calculated for 10 h post-feeding (Fig. 1).

**General performance and nutrient digestibility (Expt. 2).** Two calves (4 observations) were excluded from the statistical analysis because of feed refusals (>10% of feed allowance). At the start of the experiment, the BW of the calves was similar \((164 \pm 5.1 \text{ kg})\) for both treatments (Table 3). Feed intake did not differ between treatments, but BW gain was 46% lower \( (P < 0.05) \) for ASYN than for SYN. The apparent total tract digestibility of dry matter \((94.8\%)\), N \((90.9\%)\), energy \((95.1\%)\), crude fat \((95.9\%)\), lactose \((100.0\%)\), and ash \((78.4\%)\) and digestible nutrient intakes (data not shown) did not differ between treatments.

**Protein and energy metabolism (Expt. 2).** Urinary N excretion was greater \( (P < 0.001) \) for ASYN than for SYN, and N retention was lower \( (P < 0.01) \) for ASYN (Table 3). Similarly, the efficiency of digestible N utilization for growth was 23% lower \( (P = 0.001) \) for ASYN than for SYN. Plasma \( \alpha \)-amino N responded to feeding, but levels were not affected by treatment (data not shown). Plasma urea was higher \( (P < 0.05) \) for ASYN than for SYN, but concentrations were relatively stable during the day and differences between treatments were not time dependent (data not shown). The metabolizability of the digestible energy intake was lower \( (P < 0.05) \) for ASYN than for SYN, but ME intake did not differ between treatments (Supplemental Table 2). Heat production and fat deposition were not affected by AA synchrony. Within-day patterns for heat production and RQ did not differ between treatments.

**Indicator AA oxidation and urea production (Expt. 2).** After the 0000 h feeding, in which 25% (SYN) and 0% (ASYN) of the daily amount of crystalline Lys and Thr was supplemented, Leu oxidation was lower \( (P < 0.001) \) for ASYN than for SYN (Table 3). After the 1200 h feeding, in which 25% (SYN) and 100% (ASYN) of the daily amount of Lys and Thr was supplemented, Leu oxidation was higher \( (P < 0.001) \) for SYN than for ASYN. Daily urea production was 20% higher \( (P < 0.01) \) for ASYN than for SYN. Urea production was positively correlated with urinary N \((r = 0.72\); \( P = 0.047)\) and urea \((r = 0.77\); \( P = 0.041)\) excretion. For SYN, the diurnal pattern of urea production showed 3 to 4 peaks, with the highest rate of urea production between the 0600 and 1200 h meals. For ASYN, the circadian pattern of urea production showed only 2 distinguishable peaks, around 1200 and 2400 h, respectively (Fig. 3).

**Discussion**

**AA asynchrony and protein deposition.** The current study shows that, at equal daily AA intake, asynchrony in availability of indispensable AA increases urea production and decreases body protein deposition in growing milk-fed calves compared with synchrony. AA asynchrony reduced the efficiency by which digestible protein was deposited by 18% (Expt. 1) and 25% (Expt. 2). Early studies with AA asynchrony in rats (14–18) were inconclusive, but the findings from the current study correspond with results in several (growing) fish species, where asynchronous absorption of AA is considered as a factor for inefficient protein utilization for growth (31,32). Similar concepts may apply for other (monogastric) meal-fed animals, such as broiler breeders or gestating sows.

The experimental contrasts in this study were large, with a time lag of 6 h for AA supplementation (Expt. 1), or with

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**TABLE 3** Effects of time of oral L-Lys plus L-Thr supplementation on general performance and N utilization for growth in milk-fed calves (Expt. 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SYN</th>
<th>ASYN</th>
<th>SEM</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>157</td>
<td>171</td>
<td>5.1</td>
<td>0.104</td>
</tr>
<tr>
<td>Weight gain, g/d</td>
<td>1300</td>
<td>710</td>
<td>125.2</td>
<td>0.016</td>
</tr>
<tr>
<td>Dry matter intake, g/d</td>
<td>2198</td>
<td>2256</td>
<td>66.0</td>
<td>0.560</td>
</tr>
<tr>
<td>N intake, mg/(kg(0.75) · d)</td>
<td>1763</td>
<td>1715</td>
<td>32.7</td>
<td>0.331</td>
</tr>
<tr>
<td>Fecal N excretion, mg/(kg(0.75) · d)</td>
<td>164</td>
<td>152</td>
<td>6.0</td>
<td>0.231</td>
</tr>
<tr>
<td>Digestible N intake, mg/(kg(0.75) · d)</td>
<td>1599</td>
<td>1562</td>
<td>31.4</td>
<td>0.431</td>
</tr>
<tr>
<td>Urinary N excretion, mg/(kg(0.75) · d)</td>
<td>853</td>
<td>1014</td>
<td>17.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>N retention, mg/(kg(0.75) · d)</td>
<td>746</td>
<td>548</td>
<td>34.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Efficiency of N utilization for growth, %</td>
<td>42.3</td>
<td>31.8</td>
<td>1.51</td>
<td>0.002</td>
</tr>
<tr>
<td>Of N intake</td>
<td>46.6</td>
<td>34.9</td>
<td>1.61</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1 Data are least-square means, \( n = 6 \). ASYN, asynchrony; SYN, synchrony.
2 Calves were supplied with a milk replacer deficient in Lys and Thr at 0000, 0600, 1200, and 1800 h, and L-Lys plus L-Thr was supplemented either equally distributed over the 4 daily meals (SYN) or concentrated in the 1200 h meal (ASYN).

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**FIGURE 1** Expt. 1: Effect of time of parenteral L-Lys plus L-Thr supplementation on whole-body Leu oxidation in milk-fed calves. Values are means ± SEM, \( n = 5 \). Calves were fed with a milk replacer deficient in Lys and Thr, and L-Lys plus L-Thr was supplemented via i.v. infusion from 2 to 4 h (SYN), 0 to 2 h (PART), or 8 to 10 h (ASYN) after each feeding. The arrow represents feeding time and gray bars represent periods of parenteral AA supplementation. AA, amino acid; ASYN, asynchrony; PART, partial asynchrony; SYN, synchrony.
deficient in Lys and Thr, and L-Lys plus L-Thr was supplemented either equally distributed over the 4 daily meals (SYN) or concentrated in the 1200 h meal (ASYN). Arrows represent feeding times and supplementation of AA is indicated. AA, amino acid; ASYN, asynchrony; SYN, synchrony.

Values are means ± SEM, n = 6. Arrows represent feeding times and supplementation of AA is indicated. AA, amino acid; ASYN, asynchrony; SYN, synchrony.

supplementation in 1 vs. 4 meals (Expt. 2). Moreover, ~50% of the Lys and Thr allowance was supplied as crystalline AA. As a consequence, the effects on the efficiency of N utilization were also considerable, i.e., 7 and 11 percentage points in Expt. 1 and 2, respectively. A more subtle AA asynchrony will likely induce smaller effects but may contribute to lower dietary protein utilization under practical conditions. Nonetheless, the difference between SYN and PART in Expt. 1 did not reach significance. The latter may be related to the very low efficiency by which dietary protein was deposited in that experiment (see “Oral vs. parenteral AA supplementation”), which may have impaired the sensitivity of the N-balance technique. However, biological mechanisms can also exist for buffering AA and hence leveling out asynchrony in AA, which would corroborate results from other studies. In rats at a growth rate of 2–2.5 g/d, N retention was not inhibited by a delay of 4–6 h between a Lys-deficient diet and supplemented Lys (16,18) or by a delay of 3–5 h during Lys deprivation than other indispensable AA are when they are made equally deficient (35,40). This may be due to preventing oxidation of Lys by a transport barrier or by selective AA transport into the mitochondrial matrix, which may result in Lys incorporation in new proteins and thus turnover from protein to protein without being destroyed (35). Alternatively, the greater (+16%) AA intake for ASYN than for SYN calves with the 1200 h meal may have increased insulin release (41). This could explain the greater postprandial protein deposition by insulin-mediated inhibition of protein breakdown rather than stimulation of protein synthesis (42).

Postprandial Leu oxidation. AA oxidation consists of the disposal of the carbon skeleton through oxidation to CO2 and water, and the disposal of the amino group predominantly as urea. Therefore, we studied within-day changes in AA oxidation by measuring the oxidation of an indicator AA, i.e., [1-13C]Leu, and the 24-h kinetics of urea production.

When [1-13C]Leu was provided with the dietary AA, the postprandial oxidation of Leu was higher for ASYN than for SYN (and PART) in Expt. 1. Similar results were found in Expt. 2 after the meal without supplemented AA for ASYN (i.e., Fig. 2, 0–10 h). These results correspond with the N balance data and can be explained by a deficiency in Lys and Thr for ASYN, stimulating oxidation of imbalanced indispensable AA, including Leu. Similar oxidative responses of an indicator AA were reported when Lys was supplemented to a Lys-deficient diet in minipigs (36) and humans (37).

After supplementing AA for ASYN in Expt. 2 (i.e., Fig. 2, 12–18 h), Leu oxidation was substantially lower for ASYN than for SYN, indicating higher postprandial protein utilization for growth. This suggests that calves may spare AA from oxidation when the most limiting AA are temporarily provided in excess after a relatively short period (<24 h) of deprivation from these indispensable AA. Such a transient increase in efficiency of protein utilization involves either enhanced protein synthesis or reduced protein breakdown. It has been suggested, e.g., that the intestine can temporarily store AA by synthesis of labile proteins (38,39). In growing pigs, Ten Have et al. (39) recently showed that protein from gelatin, being deficient in Trp, was retained to a greater extent in intestinal tissues when Trp was supplemented to the diet. In addition, it has been speculated that Lys from protein breakdown in tissue pools is catabolized far slower during Lys deprivation than other indispensable AA are when they are made equally deficient (35,40). This may be due to preventing oxidation of Lys by a transport barrier or by selective AA transport into the mitochondrial matrix, which may result in Lys incorporation in new proteins and thus turnover from protein to protein without being destroyed (35). Alternatively, the greater (+16%) AA intake for ASYN than for SYN calves with the 1200 h meal may have increased insulin release (41). This could explain the greater postprandial protein deposition by insulin-mediated inhibition of protein breakdown rather than stimulation of protein synthesis (42).
maximum rate of postprandial urea production for SYN calves was at 2–3 h after feeding, whereas ASYN calves had the highest rates of urea production only at 6–8 h after the 1800 and 0600 h feedings. This may have resulted in overlap of the urea production peaks after the 2400 and 0600 h feeding for ASYN. Delayed responses of urea production to feeding, compared with Leu oxidation, correspond with findings in humans. Using combined approaches with [1-13C]Leu and [15N15N]urea, El-Khoury et al. (43) found that urea production was relatively constant during the day in meal-fed subjects, whereas Leu oxidation increased approximately 2-fold after feeding. Another study, which was similar in methodology, indicated a 4-h delay between oxidation of Leu and production of urea (44). Such differences may be due to limitations of the urea tracer technique (45). The urea dilution technique as a measure for AA oxidation has been criticized for: 1) its delay in responding to changes in AA oxidation due to the large and variable urea distribution volume; and 2) potential disturbances resulting from urea recycling. In the current study, urea production was calculated based on a constant distribution volume and sensitivity analysis on our data revealed that variation of 5% in distribution volume would change the rate of urea production only by 0.5%, which cannot explain the differences in urea production observed. We also assumed a constant rate of urea recycling during the day. Large diurnal fluctuations in urea recycling can under- or overestimate changes during certain hours within a day, but this seems unlikely, because [15N15N]urea contributed only 2.5% to total 15N in urea in cumulative 24-h urine.

Low efficiency of wheat protein utilization. Across the 2 experiments, only 28–42% of the ingested wheat protein was deposited in calves, with the highest efficiency for SYN calves in Expt. 2. This efficiency is substantially lower than that observed in other studies with calves fed skimmed milk protein [60% at 138 kg BW and 51% at 216 kg BW (46)] or whey protein [45–51% at 174 kg BW (8)]. A lower ileal digestibility of wheat protein compared with milk protein only explains 2–3% of the reduction in efficiency (47). Alternatively, the high content of dispensable AA in wheat protein, particularly glutamine (300 g/kg in wheat protein, 139 g/kg in body protein), may have contributed to the low efficiency of N utilization. This is supported by the estimated efficiency of Lys utilization in Expt. 2 (54.3%), as calculated from our N balance data and body composition data of Gerrits et al. (48), which appears considerably higher than the efficiency of total N utilization (42.3%).

Oral vs. parenteral AA supplementation. In Expt. 1, α-amino N concentrations in peripheral blood peaked between 2 and 4 h after feeding, indicating that the timing of parenteral AA supplementation in SYN calves was appropriate. Parenteral supplementation of a limiting, indispensable AA potentially increases protein deposition. For example, i.v. supplementation of Ile to pigs fed blood meal, which is deficient in Ile, reduced plasma urea and AA concentrations, indicating greater AA retention (49). Restoring AA balance by parenteral supplementation increased the efficiency of digestible protein utilization (+21%), but this response was only 63% of that of oral AA supplementation (+34%). Ultimately, parenteral supplementation resulted in a 19% lower efficiency of digestible protein utilization than oral supplementation (SYN: 37.7 vs. 46.6% for Expt. 1 and 2, respectively). Orally supplemented Lys and Thr are transferred across the splanchnic tissues together with the AA originating from dietary protein, resulting in a balanced AA supply. Parenteral supplementation will have induced relatively high levels of the supplemented AA in the systemic circulation, whereas the dietary, imbalanced AA would have dominated in splanchnic tissues. It may therefore be concluded that AA imbalance is sensed both at the level of splanchnic as well as peripheral tissues. In the splanchnic tissues, a deficiency of Lys and Thr may have triggered oxidation of dietary AA, as previously shown for Trp-deficient diets in pigs (38). Parenteral AA supplementation probably induced disproportionate AA oxidation in the splanchnic tissues, which may have altered the AA net supply to the rest of the animal. We hypothesize that especially the absence of Thr has stimulated oxidation of indispensable AA in the splanchnic tissues, because dietary Thr is normally retained for 60–80% by the portal-drained viscera, whereas the first-pass metabolism for Lys is only 30%. Moreover, dietary rather than systemic Thr is preferably utilized for splanchnic protein synthesis, resulting in a high obligatory, visceral requirement for Thr (50).

In the systemic circulation, excess Lys and Thr concentrations may induce oxidation of these AA, because fractional hepatic oxidation of individual AA is relatively constant (51). Especially the high parenteral Thr supply may have increased hepatic Thr oxidation, because high, first-pass extraction rates of Thr by intestinal tissues normally prevent large elevations of plasma Thr concentrations (52,53).

Finally, the higher feeding frequency in Expt. 2 (4 meals/d) than in Expt. 1 (2 meals/d) may also have contributed to a higher protein deposition. We previously found that increasing the frequency of feeding a whey-based diet to calves increased the efficiency of digestible protein utilization for growth from 49.1 to 54.5% (8). When this also accounts for the current study, about one-half of the difference in protein deposition between Expt. 1 and 2 can be explained by feeding frequency.

In conclusion, at identical daily intakes of all AA, introducing a time lag in availability between individual indispensable AA substantially reduced the efficiency of protein deposition in milk-fed calves. Restoring AA balance by parenteral supplementation resulted in a 19% lower efficiency of digestible protein utilization than by oral supplementation, which is likely caused by splanchnic oxidation of imbalanced AA in excess to Thr. This suggests that an AA imbalance in the splanchnic tissues may result in disproportionate AA oxidation.

Acknowledgments

Literature Cited


