Synchronizing the Availability of Amino Acids and Glucose Decreases Fat Retention in Heavy Prreruminant Calves

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Abstract

Effects of synchronizing the availability of amino acids and glucose within a day on protein and energy metabolism were studied in heavy preruminant calves. Thirty-six preruminant calves (148 ± 1.6 kg body weight) were assigned to 1 of 6 degrees of nutrient synchrony (SYN, 1–6) and to 1 of 2 meal sequences (i.e., the high-protein meal in the morning or in the evening). Calves at SYN 1 received 2 balanced meals: one at 0600 and one at 1800. Nutrient synchrony decreased stepwise from SYN 1 to SYN 6 in which calves received 85% of the daily protein supply in 1 meal. The digestible energy intakes at 0600 and 1800 were equal between treatments. Daily intakes of all nutrients and dietary ingredients were identical for all treatments. Calves were housed individually in respiration chambers. Apparent fecal nutrient digestibility and nitrogen and energy balances were measured. Apparent nutrient digestibility decreased when >71% of the dietary protein was fed in one meal. Nutrient synchrony did not affect the efficiency of digestible protein utilization in calves at a identical digestible nutrient intake. Heat production decreased from 691 to 629 kJ/(kg body weight) . d) (P < 0.05) and energy retained as fat increased from 116 to 184 kJ/(kg body weight) . d) (P < 0.01) with decreasing nutrient synchrony. Meal sequence did not affect any of the traits. In conclusion, synchronizing the availability of amino acids and glucose within a day did not increase the efficiency of protein utilization but substantially decreased fat retention in heavy preruminant calves. J. Nutr. 136: 2181–2187, 2006.

Introduction

Dietary protein is efficiently utilized for body protein deposition in growing animals and man. During the first 4 wk of life, ~80% of the milk protein provided above that required for maintenance is deposited as body protein in rats, pigs, sheep, and man (1). After weaning, slightly lower values are reached for the marginal efficiency of digestible protein utilization in growing rats and pigs (2,3). In heavy preruminant calves, however, the marginal efficiency of protein utilization for growth is very low (~30%) as compared with other species (4). Several potential mechanisms to explain this lower efficiency, i.e., 1) a protein-energy imbalance (5), 2) an imbalanced amino acid profile (6), and 3) the use of amino acids for ammonia detoxification (7), have been explored and discussed, but none of these were responsible for the inefficient protein utilization in preruminant calves (4). We suggested that the problem may be of multifactorial origin, and that a lack of nutrient synchrony may be involved (4).

A decreased nutrient synchrony, i.e., a nearly complete separation of protein and carbohydrate intake in time, was shown to decrease the efficiency of protein utilization from 57 to 47% of the digestible protein intake in growing pigs (J. J. G. C. van den Borne, J. W. Schrama, M. J. W. Heetkamp, M. W. A. Verstegen, and W. J. J. Gerrits, unpublished results). In preruminant calves, nutrient synchrony may be decreased when milk replacers are based on skimmed milk protein. Casein, representing 80% of the skimmed milk protein, coagulates in the abomasum and is released gradually during the day, whereas the absorption of glucose and galactose peaks within 1 h postfeeding (8). This separation of amino acid and monosaccharide availability in time can result in within-day deficiencies or excesses of amino acids relative to glucose and may prevent an efficient protein deposition in milk-fed calves. Therefore, we hypothesize that a lack of postabsorptive synchrony between nutrients restricts the efficiency of protein utilization in preruminant calves.

When compared with amino acids, the body has only a limited capacity to store glucose. With evidence that diurnal variation in metabolic processes is related to physical activity (9,10), the body may prefer glucose oxidation over amino acid oxidation to generate energy during the day. The sequence (SEQ) of a high protein and a high carbohydrate meal may therefore affect protein retention. Moreover, we speculate that

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the response of metabolism to a separation of glucose and amino acid availability within a day depends on the degree of nutrient synchrony.

We hypothesized that, at equal daily nutrient intakes, a decreased synchrony of the protein and lactose supply over 2 meals would decrease the efficiency of protein utilization in heavy preruminant calves. The aim of the experiment was to test the linearity of these effects and to study the impact of the hypothesized reduction in protein deposition on fat deposition. In addition, we examined the effect of the sequence of the high protein and high lactose meals on protein utilization and energy partitioning.

Materials and Methods

Animals and housing. Thirty-six male, Holstein Friesian calves were used in 18 trials of 2 calves each. Using a 6 × 2 factorial design, calves were assigned to 1 of 6 degrees of nutrient synchrony (SYN 1–6) and to 1 of 2 meal sequences (SEQ A or B). Allocation of SYN and SEQ to calves was balanced in time. Daily nutrient intakes were identical for all treatments. The degree of synchrony determined the distribution of protein and lactose over the 2 daily meals. Calves at SYN 1 were fed the daily amount of protein and lactose equally divided over both meals, whereas calves at SYN 6 received 85% of the daily protein supply in one meal and the remaining 15% in the other meal (Table 1). Other treatments (SYN 2–5) were intermediate. Protein and lactose were exchanged based on digestible energy, which resulted in identical digestible energy intakes in morning and evening meals across treatments. Protein and lactose supply were step-wise less synchronous from treatment 1 to 6. For calves at meal sequence A (SEQ A), the high protein meal was fed in the morning and the high lactose meal in the evening. For SEQ B, this sequence was reversed.

Calves arrived at the experimental facility at the age of 2 wk and were raised for 10 wk on a commercial milk replacer, after which they were adapted to experimental treatments and housing conditions for 4 wk. Harnesses for the fecal collection bags were attached 5 d before the start of the experiment. The experimental period consisted of a 7-d balance period. Calves were individually housed in 1 of 2 identical climatic respiration chambers set to measure 2.5 × 1.5 × 2.0 m (L × W × H). Within each chamber, calves were housed in metabolic cages (1.85 × 0.75 m). Calves 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 0600 to 1830) and 11 h of partial darkness (6 lx). The experiment was approved by the Ethical Committee of Wageningen University.

### TABLE 1  Experimental treatments and distribution of the nutrient intake over 2 daily meals

<table>
<thead>
<tr>
<th>Treatment (SYN)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON⁴</td>
<td>50</td>
<td>57</td>
<td>64</td>
<td>71</td>
<td>78</td>
<td>85</td>
</tr>
</tbody>
</table>

### TABLE 2  Ingredient and analyzed nutrient composition of the 2 basal diets

<table>
<thead>
<tr>
<th>Item</th>
<th>High protein diet</th>
<th>High lactose diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg</td>
<td>g/kg</td>
</tr>
<tr>
<td>Ingredient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whey protein concentrate</td>
<td>464.3</td>
<td>68.6</td>
</tr>
<tr>
<td>Soy oil</td>
<td>209.7</td>
<td>175.7</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>52.4</td>
<td>43.9</td>
</tr>
<tr>
<td>Lactose</td>
<td>214.6</td>
<td>662.3</td>
</tr>
<tr>
<td>Premix⁵</td>
<td>54.6</td>
<td>45.8</td>
</tr>
<tr>
<td>Iron-supplement</td>
<td>4.4</td>
<td>3.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>High protein diet</th>
<th>High lactose diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>985.5</td>
<td>992.5</td>
</tr>
<tr>
<td>Crude protein</td>
<td>364.5</td>
<td>62.1</td>
</tr>
<tr>
<td>Crude fat</td>
<td>217.8</td>
<td>178.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>211.4</td>
<td>617.1</td>
</tr>
<tr>
<td>Ash</td>
<td>62.0</td>
<td>36.3</td>
</tr>
</tbody>
</table>

⁴ CON, contribution of the high protein meal to the daily protein supply (50% = two identical, balanced meals; 100% = all daily protein in one meal).

⁵ Treatments are presented for meal sequence A. For meal sequence B, the nutrient intake in the morning and evening meal was reversed.

Diets and feeding. Calves were fed according to their metabolic body weight (kg⁰.⁷⁵), and feed supply was adjusted daily for a projected gain of 1000 g/d. Metabolizable energy requirements for maintenance (MEₘ) were assumed to be 460 kJ/(kg⁰.⁷⁵ · d) (5,11). The feeding level was 2.0 × MEₘ. Two basal diets were prepared for the 5 synchronous treatments (SYN 6) and diets for the other 5 treatments (SYN 1–5) were created by mixing the 2 basal diets in a ratio appropriate to the experimental treatments indicated in Table 1. All calves received 9.3 g crude protein, 9.1 g crude fat, and 20.1 g lactose per kg⁰.⁷⁵ daily. The ingredients and analyzed nutrient composition of the basal diets are shown in Table 2. Milk replacer was reconstituted with water (140 g/L) and supplied at a temperature of ~40°C in a bucket. Roughage was not supplied. Calves were fed individually at 0600 and 1800. Calves were allowed 15 min to consume the meal.

Measurements. A balance trial was performed to measure apparent fecal nutrient digestibility and energy and protein retention. Feces were collected quantitatively from plastic bags that were harnessed to the calves, and the collections were stored at ~20°C. For each balance period, morning and evening feces were pooled separately over days and pH was measured in morning and evening feces. After mixing the morning and evening feces, pH was measured again and feces were sampled for further analyses. Urine was collected in a pit containing 50 mL of 4.5 mol/L sulphuric acid. Aerial NH₃ was quantitatively trapped in 4.5 mol/L sulphuric acid and in water that condensed on the heat exchanger. Feed refusals were collected, registered, and frozen at ~20°C pending further analyses. Feed was sampled for each balance period and stored at 4°C pending further analyses.

To determine dry matter content, feed refusals and fresh feces were freeze-dried; feed samples were vacuum-dried at 80°C; and air-dried feces were dried in a forced-air oven at 103°C. All samples were dried to a constant weight according to ISO 6496 (12). After freeze-drying, feces were ground to pass through a 1 mm screen and kept for analyses. Nitrogen content was measured in fresh feed, feed refusals, fresh feces, urine, sulphuric acid containing aerial NH₃, and water from condensed aerial NH₄ according to ISO 5983 (13). For calculations, nitrogen in aerial NH₄ was assumed to be of urinary origin. Crude fat content was determined in feed and in freeze-dried feces after acid hydrolysis according to ISO 6492 (14). Crude ash content was determined in feed and in freeze-dried feces. Samples were carefully incinerated in a muffle furnace.
furnace by slowly increasing the temperature from 20°C to 550°C to prevent foaming, and subsequent incineration occurred according to ISO 5984 (15). Lactose content was analyzed enzymatically in feed and in freeze-dried feces (Enzytec, Difffchamb Biocontrol). Gross energy content was analyzed in feed, freeze-dried feces, and freeze-dried urine using adiabatic bomb calorimetry (IKA-calorimeter C7000) according to ISO 9831 (16). All analyses were carried out in duplicate, except nitrogen content in urine, which was determined in triplicate.

Gas exchange was measured in 6-min intervals by measuring the exchange of oxygen, carbon dioxide, and methane as described by Verstegen et al. (17). Posture of calves was measured every minute by infrared beam interruption and was expressed as lying (i.e., lying during the complete 6-min interval) or nonlying (i.e., standing for ≥1 min of the 6-min interval). Physical activity was recorded with a radar-Doppler device according to the method described by Wenk and Van Es (18).

Calculations. For each balance period, intake of metabolizable energy per calf was calculated as the difference between digestible energy intake and the sum of urinary energy losses and methane production. From the gaseous exchanges, heat production (H$_{\text{tot}}$) was calculated according to the formula of Brouwer (19). Energy retention was calculated by subtracting H$_{\text{tot}}$ from metabolizable energy intake. Retention of nitrogen was calculated from N in feed and N in excreta. Energy retained as protein was derived from retained N, assuming 160 mg N and 23.6 kJ/g of protein. Energy retained as fat was calculated by subtracting energy retained as protein from overall energy retention. The respiratory quotient (RQ) was calculated as the CO$_2$ production divided by the O$_2$ consumption. For each calf within a balance period, the energy costs per unit of physical activity were estimated by regression of physical activity against heat production and subsequent calculation of heat production for physical activity (H$_{\text{act}}$) as described by Van den Borne et al. (20). Balance period means were calculated for all variables and hourly means were calculated for H$_{\text{tot}}$, H$_{\text{act}}$, and RQ.

Statistical analysis. The effects of the degree of nutrient synchrony and the interaction between the degree of nutrient synchrony and meal sequence on apparent fecal digestibilities, energy, and nitrogen balance parameters, circadian rhythms of heat production traits, and RQ were analyzed in a mixed model, using the degree of nutrient synchrony as a regressor, according to the following model:

$$Y_{ij} = \mu + \beta_1 \times X_i + \beta_2 \times S \times X_i + e_{ij},$$

where $Y_{ij}$ is the dependent variable over the whole period (or the hourly mean), $\mu$ is the average intercept, $\beta_1$ is the effect of the degree of nutrient synchrony expressed as percentage of the daily protein intake in the high protein meal, $\beta_2$, is the interaction between degree of nutrient synchrony and meal sequence, $X_i$ is the degree of nutrient synchrony (expressed as percentage of the daily protein intake in the high protein meal) for calf $j$, $e_{ij}$ = error term, $i$ = 1, 2, and $j$ = 1 … 18. Treatment effects on H$_{\text{tot}}$, H$_{\text{act}}$, and RQ were tested for each hour separately. Differences were considered significant at $P < 0.05$. The SAS software package version 9.1 was used in all statistical evaluations. Results are presented as means ± SEM.

Results

General. The initial body weight (148 ± 1.6 kg) and daily gain (1209 ± 46.3 g) did not differ between treatments (Table 3). Two calves were excluded from the analysis, because of feed refusals or illness. Feed intake was similar for all treatments. Digestive problems (i.e., diarrhea) occurred for calves at SYN 5 and 6. Compared with feces of calves at SYN 1 to 4, feces of calves at SYN 5 and 6 were characterized by a lower dry matter content (11.9 vs. 18.8%; P < 0.01) and a reduced pH (5.9 vs. 7.8; P < 0.01) (Fig. 1). Feces collected after the morning meal did not differ in pH from feces collected after the evening meal (data not shown). Consequently, calves at SYN 5 and 6 had a lower (P < 0.001) digestible nutrient intake than calves at SYN 1–4. Therefore, linear regression was performed separately for SYN 1–6 and SYN 1–4. The meal sequence did not affect the mean weekly balance traits, and interactions between SYN and SEQ were not present. Results are therefore presented as pooled data without differentiating for SEQ A and SEQ B.

Digestibility. Apparent fecal nutrient digestibility, except for ash, decreased with decreasing nutrient synchrony (P < 0.01). This decrease was due to low nutrient digestibilities for SYN 5 and 6. Nutrient digestibility was not affected when only SYN 1–4 were taken into account. Accordingly, the daily digestible intake of individual nutrients (data not shown) was identical for SYN 1–4 but substantially lower for SYN 5 and 6 (P < 0.01).

Nitrogen metabolism. Nitrogen intake did not differ between treatments and fecal nitrogen excretion was not affected (SYN

### TABLE 3

Effects of nutrient synchrony on initial body weight, feed intake, daily gain, and apparent fecal nutrient digestibility in heavy preruminant calves.

<table>
<thead>
<tr>
<th>Treatment (SYN)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>CON 50–85</th>
<th>CON 50–71</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON$^2$</td>
<td>50</td>
<td>57</td>
<td>64</td>
<td>71</td>
<td>78</td>
<td>85</td>
<td>SEM</td>
<td>SEM</td>
</tr>
<tr>
<td>Observations, n</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Initial body weight, kg</td>
<td>147</td>
<td>147</td>
<td>145</td>
<td>150</td>
<td>143</td>
<td>155</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Feed intake, g/d</td>
<td>2007</td>
<td>2031</td>
<td>1995</td>
<td>2063</td>
<td>1955</td>
<td>2109</td>
<td>17.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Feed intake, g/(kg$^{\text{PD}}$ × d)</td>
<td>46.5</td>
<td>47.1</td>
<td>46.7</td>
<td>47.0</td>
<td>46.8</td>
<td>47.0</td>
<td>0.11</td>
<td>0.007</td>
</tr>
<tr>
<td>Daily gain, g</td>
<td>1208</td>
<td>1307</td>
<td>1297</td>
<td>1387</td>
<td>941</td>
<td>1133</td>
<td>46.3</td>
<td>-6.0</td>
</tr>
</tbody>
</table>

**Digestibility**

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>SEM</th>
<th>b$^4$</th>
<th>P-value$^4$</th>
<th>SEM</th>
<th>b</th>
<th>P-value$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td></td>
<td>0.31</td>
<td>-1.0</td>
<td>&lt;0.001</td>
<td>0.22</td>
<td>0.1</td>
<td>0.706</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td>0.78</td>
<td>-2.1</td>
<td>&lt;0.001</td>
<td>0.57</td>
<td>-0.2</td>
<td>0.796</td>
</tr>
<tr>
<td>Ash</td>
<td></td>
<td>0.98</td>
<td>-0.6</td>
<td>0.464</td>
<td>1.24</td>
<td>1.1</td>
<td>0.510</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td>0.52</td>
<td>-1.3</td>
<td>0.002</td>
<td>0.44</td>
<td>-0.5</td>
<td>0.356</td>
</tr>
<tr>
<td>Lactose</td>
<td></td>
<td>2.73</td>
<td>-5.5</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td>0.38</td>
<td>-1.3</td>
<td>&lt;0.001</td>
<td>0.25</td>
<td>-0.3</td>
<td>0.405</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SEM.
$^2$ CON, contribution of the high protein meal to the daily protein supply (50% = two identical, balanced meals; 100% = all daily protein in one meal).
$^3$ Regression coefficient γ = a + b × x, representing the change in response parameter per percentage increase of the contribution of the high protein meal to the daily protein supply.
$^4$ Probability for test if the regression coefficient (b) equals 0. Effects of meal sequence and interactions between meal sequence and degree of nutrient synchrony were nonsignificant and were therefore excluded from the model.

Nutrient synchrony in preruminant calves

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[Downloaded from https://academic.oup.com/jn/article-abstract/136/8/2181/4664798 by guest on 25 January 2019]
Effect of nutrient synchrony on fecal dry matter content and fecal pH in heavy preruminant calves. Asterisks indicate difference between 78% of the daily protein in the high protein meal (SYN 5) and 85% of the daily protein in the high protein meal (SYN 6) versus 50–71% of the daily protein in the high protein meal (SYN 1–4) (**P < 0.01). Values are means ± SEM, n = 5 (SYN 1–2) or n = 6 (SYN 3–6).

1–4) by decreasing nutrient synchrony (Table 4). Therefore, digestible nitrogen intake was identical between treatments (P < 0.01; SYN 1–4). Urinary nitrogen excretion and nitrogen retention were not affected by the degree of nutrient synchrony. The degree of nutrient synchrony also did not affect nitrogen retention when expressed as percentage of either nitrogen intake or digestible nitrogen intake.

Energy metabolism. When considering treatments SYN 1 to SYN 4, intakes of gross, digestible, and metabolizable energy were not affected by the degree of nutrient synchrony (Table 5). Heat production, however, decreased by 62 kJ/(kg^0.75·d) from SYN 1 to SYN 4 (P < 0.01). Activity-related heat production was not affected by nutrient synchrony, but activity-corrected heat production decreased by 52 kJ/(kg^0.75·d) from SYN 1 to SYN 4 (P < 0.01). Energy retention increased gradually from 6 (SYN 3–6).

Protein metabolism. In contrast to our hypothesis, nitrogen retention in heavy preruminant calves was not reduced by a decreased nutrient synchrony. The degree of nutrient synchrony also did not affect nitrogen utilization even increased numerically with decreasing nutrient synchrony: from 45.9% for SYN 1 to 49.7% for SYN 4 (P = 0.138). This implies that an asynchronous nutrient availability does not explain the low efficiency of protein utilization in heavy milk-fed calves. Nutrient availability was more asynchronous in the present study than in practical diets containing skimmed milk protein. In the present study, the molar ratio between amino acids and lactose varied from 0.6 in the high lactose meal to 2.7 in the high protein meal for SYN 4. This ratio was probably slightly lower in portal blood, because absorbed amino acids are usually oxidized to a larger extent by intestinal tissues than absorbed glucose (26). The asynchrony was, however, substantially higher when skimmed milk protein was fed to heavy preruminant calves, which resulted in a molar ratio of amino acids relative to glucose of 1.8 (8).

The lack of response of nitrogen retention to an increased separation of protein and carbohydrate intake over time contrasts with effects in growing pigs. In pigs, a virtual complete separation of protein and carbohydrate intake over 2 daily meals substantially decreased the efficiency of nitrogen utilization (J. J. G. C. van den Borne, J. W. Schrama, M. J. W. Heetkamp, M. W. A. Verstegen, and W. J. J. Gerrits, unpublished results). Similar effects were found in growing rats (27) and growing lambs (using volatile fatty acids instead of carbohydrate) (28). A partial separation of protein and carbohydrate intake decreased protein utilization in growing boys (29) but not in growing pigs (30,31). It can therefore be concluded that protein metabolism in preruminant calves responded differently to a decreased nutrient synchrony compared with most other studies in the literature. Several reasons for this discrepancy can be suggested.

Discussion

General. The high moisture content and decreased pH in feces of calves at the 2 most asynchronous treatments (SYN 5 and 6) suggest that osmotic diarrhea occurred in those calves. For an appropriate assessment of effects of nutrient synchrony on nitrogen utilization and energy partitioning, treatments should be compared at identical digestible nutrient intakes. Therefore, this discussion focuses mainly on the effects observed from treatments SYN 1 to SYN 4, with a maximal experimental contrast of 71 and 32% of the daily protein and lactose intakes, respectively, in one and the remainder in the other meal (SYN 4).

Digestibility. Apparent nutrient digestibility was generally high and in accordance with values reported in other studies (5,21). Decreasing nutrient synchrony (SYN 1–4) did not affect nutrient digestibility. When protein and carbohydrate intake were more separated, SYN 5 and 6, nutrient digestibility decreased. Apparent fecal lactose digestibility was incomplete for SYN 5 (93%) and SYN 6 (78%), suggesting the occurrence of osmotic diarrhea. Weijers and Van de Kamer (1965) stated that, in man, a low fecal pH in combination with a low dry matter content usually indicates carbohydrate fermentation, whereas loose feces in combination with a high pH, is related to putrefactive diarrhea (22). In preruminant calves, the relation between the origin of diarrhea and the associated fecal characteristics seems to be less clear (23,24). Nonetheless, an excessive daily lactose intake (> 10 g/kg BW·d) in young milk-fed calves resulted in diarrhea and a low fecal pH (25). Mean daily lactose intake in the present study was only 6 g/kg BW·d and diarrhea occurred if > 4 g/kg BW was provided in 1 of 2 daily meals. Therefore, an extreme separation of protein and carbohydrate intake within a day affects digestion and gut health in heavy preruminant calves.
Effects of nutrient synchrony on protein metabolism in heavy preruminant calves

First, the response of protein retention to an asynchronous nutrient availability can be nonlinear and thus become evident only when protein and carbohydrate intake are more separated. The degree of nutrient synchrony decreased stepwise, but did not ultimately result in a complete separation of the protein and carbohydrate intake in calves. The presence of lactose in the high protein diet may, to some extent, have prevented amino acids from being oxidized to meet the energy requirements for maintenance. Lactose in the high protein diet may also have spared amino acid utilization for gluconeogenesis.

Second, insulin resistance can explain the absence of an increase in protein retention with increasing nutrient synchrony in heavy preruminant calves. Insulin sensitivity in preruminant calves decreases markedly toward the end of the fattening period (32,33) and possibly results in glucosuria. Substantial amounts of glucose were detected in urine of calves in the current study (data not shown), indicating insulin resistance. In insulin-sensitive animals, however, insulin (strongly induced by glucose availability) stimulates protein synthesis provided that amino acids are present. The effects of insulin and amino acids in stimulating protein synthesis are synergistic and protein synthesis in muscle is unchanged if only glucose is supplied (34).

**TABLE 4** Effects of nutrient synchrony on protein metabolism in heavy preruminant calves

<table>
<thead>
<tr>
<th>Treatment (SYN)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>CON 50–85</th>
<th>CON 50–71</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>57</td>
<td>64</td>
<td>71</td>
<td>78</td>
<td>85</td>
<td>SEM  b^2</td>
<td>P-value^4</td>
</tr>
<tr>
<td>Nitrogen intake (NI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.9 0.1</td>
<td>0.888 3.8 0.6</td>
</tr>
<tr>
<td>Fecal nitrogen</td>
<td>98</td>
<td>107</td>
<td>111</td>
<td>107</td>
<td>201</td>
<td>144</td>
<td>8.3 2.1</td>
<td>0.002 7.2 0.5</td>
</tr>
<tr>
<td>Digestible nitrogen intake (DNI)</td>
<td>1386</td>
<td>1401</td>
<td>1394</td>
<td>1395</td>
<td>1279</td>
<td>1359</td>
<td>9.6 −2.0</td>
<td>0.012 8.1 0.1</td>
</tr>
<tr>
<td>Urinary nitrogen</td>
<td>749</td>
<td>752</td>
<td>693</td>
<td>701</td>
<td>735</td>
<td>713</td>
<td>10.1 −0.8</td>
<td>0.345 14.1 −2.9</td>
</tr>
<tr>
<td>Nitrogen retention</td>
<td>637</td>
<td>648</td>
<td>701</td>
<td>694</td>
<td>544</td>
<td>646</td>
<td>14.3 −1.3</td>
<td>0.355 15.9 3.0</td>
</tr>
</tbody>
</table>

Efficiency of nitrogen utilization as percentage of NI:

| as percentage of NI | 43.0 43.0 | 46.6 46.2 | 36.8 43.0 | 0.96 −0.08 | 0.312 1.08 0.18 | 0.200  |
| as percentage of DNI | 45.9 | 46.3 | 50.3 | 49.7 | 42.5 | 47.5 | 0.96 −0.02 | 0.765 1.04 0.21 | 0.120  |
Metabolizable energy intake increased 0.05) between SYN 1 to SYN 4. As a result, energy retention increased by 77 kJ/(kg\(^{0.75}\) · d) with a decreasing degree of nutrient synchrony (SYN 1–4). Protein and fat retention contributed 11 and 89% of the total increase in energy retention, respectively.

The increased fat deposition rate in asynchronously fed calves is expected to originate from an increased incorporation of dietary fatty acids in adipose tissue rather than from an increased de novo fatty acid synthesis. Synthesis of fatty acids from glucose is probably less important in preruminant calves than in pigs, because the RQ did not exceed 0.91 within the day for calves at SYN 4, whereas it exceeded 1.00 (viz. indication of net fatty acid synthesis) for several hours per day in growing pigs after a high starch diet (40). Furthermore, there is evidence that preruminant calves may not even be capable of de novo fatty acid synthesis from glucose. Potential ruminants have, for example, low activities of citrate lyase and NADP-malate dehydrogenase (41). A reduced de novo fatty acid synthesis is consistent with the observation that daily fat intake exceeded daily fat deposition by 2-fold, allowing the possibility that fat deposition completely originated from dietary fat. Within the present study, RQ increased (P < 0.05) from SYN 1 to SYN 4, which also indicates a higher contribution of glucose relative to fatty acid oxidation and allowing more fat to be deposited. The low variability in RQ within a day (Fig. 2B) possibly originates from the high dietary fat content, but also the previously mentioned peculiarities of glucose metabolism (i.e., insulin resistance and low de novo fatty acid synthesis) may contribute to a lack of flexibility to select substrates for oxidation.

Figure 2  Effects of synchronous (SYN 1; n = 5) or asynchronous (SYN 4; n = 6) availability of amino acids and glucose in heavy preruminant calves on the circadian rhythms of heat production (H\(_{tot}\)) and activity related heat production (H\(_{act}\)) [A], and the respiratory quotient (B). Because interactions with meal sequence were not significant, data were pooled over meal sequences and presented as the high protein meal in the morning and the high lactose meal in the evening. Asterisks indicate differences (P < 0.05) between treatments. Arrows represent feeding times. Results are expressed as means ± SEM.

calves. Alternatively, it can be speculated that protein retention was increased with decreasing nutrient synchrony. A higher increase of plasma free amino acid levels (i.e., after the high protein meal) may be required to stimulate muscle protein synthesis in insulin-resistant animals. In elderly women (35) and rats (36), which often have a decreased insulin sensitivity (37,38), protein utilization increased when the majority of the daily protein intake was consumed in a single meal (i.e., separated from carbohydrate intake) compared with when it was distributed over 4 meals.

Third, fatty acid oxidation has probably contributed significantly to the energy requirements for maintenance. The fat content in milk replacer diets is generally high (~20%) and fatty acid oxidation can have limited oxidation of amino acids to provide ATP for maintenance processes after the high protein meal. The source of nonprotein energy (fat or carbohydrates) does probably not affect protein retention in preruminant calves (39).

In summary, nutrient synchrony in heavy preruminant calves did not increase protein retention as hypothesized and as observed in other species. The incomplete separation of protein and carbohydrate, insulin resistance, and a high dietary fat content may explain the lack of effect in this study.

Energy metabolism. Metabolizable energy intake increased numerically by 15 kJ/(kg\(^{0.75}\) · d) and H\(_{tot}\) decreased by 62 kJ/(kg\(^{0.75}\) · d) (P < 0.05) when nutrient synchrony decreased from SYN 1 to SYN 4. As a result, energy retention increased by 77 kJ/(kg\(^{0.75}\) · d) with a decreasing degree of nutrient synchrony (SYN 1–4). Protein and fat retention contributed 11 and 89% of the total increase in energy retention, respectively.

The increased fat deposition rate in asynchronously fed calves is expected to originate from an increased incorporation of dietary fatty acids in adipose tissue rather than from an increased de novo fatty acid synthesis. Synthesis of fatty acids from glucose is probably less important in preruminant calves than in pigs, because the RQ did not exceed 0.91 within the day for calves at SYN 4, whereas it exceeded 1.00 (viz. indication of net fatty acid synthesis) for several hours per day in growing pigs after a high starch diet (40). Furthermore, there is evidence that preruminant calves may not even be capable of de novo fatty acid synthesis from glucose. Potential ruminants have, for example, low activities of citrate lyase and NADP-malate dehydrogenase (41). A reduced de novo fatty acid synthesis is consistent with the observation that daily fat intake exceeded daily fat deposition by 2-fold, allowing the possibility that fat deposition completely originated from dietary fat. Within the present study, RQ increased (P < 0.05) from SYN 1 to SYN 4, which also indicates a higher contribution of glucose relative to fatty acid oxidation and allowing more fat to be deposited. The low variability in RQ within a day (Fig. 2B) possibly originates from the high dietary fat content, but also the previously mentioned peculiarities of glucose metabolism (i.e., insulin resistance and low de novo fatty acid synthesis) may contribute to a lack of flexibility to select substrates for oxidation.

Noticeably, the increased fat retention seems not to be related to one particular meal in asynchronously fed calves, because the reduced H\(_{tot}\) with increasing nutrient asynchrony occurred after both meals (Fig. 2A). The increase in RQ after ingesting both daily meals was steeper for SYN 1 than for SYN 4 (Fig. 2B). This was expected for the high protein meal, but not for the high lactose meal. Differences in the kinetics of digestive processes (gastric emptying, digestion, and absorption) or in postabsorptive kinetics of metabolic processes (release of nutrients into the portal vein, intracellular nutrient uptake, and oxidation) may be involved. More detailed studies about quantitative interactions between glucose and fat metabolism in preruminant calves may explain the mechanism of an increased fat deposition.

In conclusion, an extreme decrease in nutrient synchrony in preruminant calves was shown to cause diarrhea and to reduce nutrient digestibility. Supplying 71 and 32% of the daily protein and lactose intakes, respectively, in one meal and the remainder in the other meal could be realized without negatively affecting nutrient digestibility. Separating protein and carbohydrate intake within a day to this extent did not reduce the efficiency of digestible nitrogen utilization for protein retention in heavy preruminant calves. Remarkably, heat production decreased and fat retention increased substantially with a decreasing degree of nutrient synchrony. With the considerable experimental contrast realized in this study, it is unlikely that an asynchronous nutrient availability contributes to the low efficiency of protein utilization often observed in heavy preruminant calves. The findings from this study may be used in devising new feeding strategies for preruminant calves when increased rates of fat deposition are desired.

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Literature Cited


