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Reviewing the low efficiency of protein utilization in heavy preruminant calves – a reductionist approach

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(Received 23 September 2005; accepted 10 January 2006)

Abstract – The efficiency of protein utilization for growth in preruminant calves is decreasing with increasing body weight. In contrast to calves weighing less than 100 kg of body weight, heavy preruminant calves do not respond in protein retention to an increased intake of indispensable amino acids in dose-response studies. The marginal efficiency of protein utilization is low compared with pigs and milk-fed lambs at a similar stage of maturity. A reductionist approach was taken to perceive the potential mechanisms for the low protein utilization in preruminant calves. Neither an imbalance in the dietary protein to energy ratio nor a single limiting indispensable amino acid was responsible for the low efficiency. Also, amino acids were not specifically used to detoxify ammonia. Alternative hypotheses to explain the low efficiency are discussed and result in (i) a reduced post-absorptive supply of amino acids: e.g. by fermentation of milk in the (premature) rumen or preferential amino acid utilization by specific tissues; or (ii) a reduced post-absorptive amino acid utilization: e.g. by decreased insulin sensitivity, utilization of amino acids for gluconeogenesis or an asynchronous nutrient supply. In conclusion, several mechanisms for the low efficiency of protein utilization in heavy preruminant calves were excluded. Other physiological processes which are potentially involved remain to be studied, because the large potential for improving protein utilization in heavy preruminant calves asks for further exploration of their amino acid metabolism.

calf / veal / protein metabolism / efficiency / amino acids / preruminant

1. INTRODUCTION

In contrast to the comprehensive work on protein digestion in milk-fed calves [1–5], the post-absorptive utilization of amino acids has only been scarcely investigated. This lack of attention is rather surprising, since only about 10% of the ingested nitrogen is lost with feces, and an additional 40–50% with urine. Furthermore, the efficiency

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of protein utilization decreases with increasing body weight (BW) in preruminant calves. Although this reduction is in accordance with the decreasing amino acid utilization for protein accretion with increasing stage of maturity [6], the efficiency in heavy preruminant calves is extremely low compared with other species. In pigs, for example, gross efficiencies of protein utilization of 60% up to as high as 81% were reported [7–10]. For a sound comparison of efficiencies, however, different species should be compared at a similar stage of maturity, and marginal efficiencies, rather than gross efficiencies, should be compared. The marginal efficiency expresses the response of protein deposition rate to increased digestible protein intake, and consequently excludes digestion inefficiency and amino acid requirements for maintenance. It has the additional advantage of showing less between-experiment variation than the gross efficiency and is thus more suitable for comparison across literature sources.

Figure 1 shows the marginal efficiency with which digestible protein, or the first limiting amino acid, was used for protein gain in pigs, preruminant lambs and preruminant calves during (early) development. The protein mass, as the percentage of protein mass at maturity, is used to indicate the stage of maturity of each species. Assumptions for the mature protein mass were based on literature values for pigs [11], sheep [12, 13], and Holstein-Friesian bulls [14]. Marginal efficiencies vary substantially between studies and even within species at a similar stage of maturity. It is, however, illustrated that the marginal efficiency of protein retention is remarkably low in milk-fed calves as compared to pigs and milk-fed lambs. The differences are more pronounced with increasing protein mass. An exception in the figure are the control pigs of Krick et al. [15] which had a marginal efficiency of only 32%, but an energy constraint to protein deposition could not be excluded in that study.

This paper reviews the work, performed at the TNO Nutrition and Food Research Institute, ILOB, Wageningen, The Netherlands, over the last twenty years (in part unpublished), in which the low efficiency
of protein utilization in heavy preruminant calves was established. Following a reductionist approach and based on experimental evidence, various factors are excluded as reasons for the low efficiency. Alternative mechanisms are presented and discussed.

2. THE AGE DEPENDENCY OF PROTEIN UTILIZATION IN PRERUMINANT CALVES

Although Donnelly and Hutton [30] found a marginal efficiency of protein utilization of only 45% in young calves (40–70 kg BW), other studies reported substantially higher marginal efficiencies of 66–69% [29, 32]. Furthermore, from various sources [33–35], gross efficiencies of digestible protein utilization of 60–68% could be calculated for preruminant calves of similar BW.

The clear response of protein gain to increased protein intake in young calves was confirmed in amino acid requirement studies [36, 37]. The amino acids studied were lysine, threonine and methionine+cysteine, and all experiments were performed with preruminant, male Holstein-Friesian × Dutch-Friesian crossbreeds, measuring nitrogen balance as the response variable to increased intakes of the amino acid of interest. Skimmed milk protein and crystalline amino acids were used as the only protein sources. The dietary crude protein content depended on the BW range of the calves and decreased from 220 g·kg⁻¹ for calves between 50 and 70 kg BW to 180 g·kg⁻¹ for the calves between 220 and 250 kg BW. Skimmed milk protein provided up to 160 g·kg⁻¹ of the diet for calves between 50 and 70 kg, and up to 120 g·kg⁻¹ for the calves between 220 and 250 kg. The remaining protein (60 g·kg⁻¹) was provided as crystalline amino acids, except for the amino acid of the study which was added in graded levels. In total, crystalline amino acids provided maximally 70 g·kg⁻¹ of the diet (a mixture of indispensable and dispensable amino acids). A quadratic response of nitrogen balance to increased intakes of lysine and methionine+cysteine was obtained for preruminant calves between 50 and 70 kg BW (P < 0.01) [38].

Similar studies were performed in heavy preruminant calves between 120 and 260 kg BW [36, 37]. Interestingly, responses of nitrogen balance to increased intakes of lysine and methionine+cysteine in heavy preruminant calves (120–260 kg BW) were absent (Fig. 2). Nitrogen balance only responded (slightly) to lysine intake in the range of 120–140 kg BW (P < 0.05). Possible explanations for the lack of response in heavy preruminant calves to an increasing amino acid intake include the following: (1) the use of large quantities of dispensable crystalline amino acids in these studies, and (2) a protein-energy imbalance in these amino acid requirement studies. Crystalline amino acids were included because skimmed milk powder was used as the main protein source, complicating a design of diets limited in specific indispensable amino acids. The possibility of an imbalanced protein to energy ratio was subsequently studied in serial slaughter experiments [31].

3. PROTEIN TO ENERGY IMBALANCE

In pigs, much more than in preruminant calves, research efforts have concentrated on the interaction between dietary protein and dietary energy [39–41]. At low protein intakes, growing pigs will preferentially use absorbed amino acids with a high efficiency for deposition in the body. With increasing intakes, protein retention will increase almost linearly up to the point at which other factors than protein intake limit protein retention. When maximum protein deposition has not been reached, energy intake is the limiting factor at this breakpoint in the curve. Any further increase in protein intake would not result in additional protein retention. Following the same approach for preruminant calves, two slaughter experiments were conducted to quantify...
the responses of protein and fat deposition rates to changes in protein and protein-free energy intake in preruminant calves from 80 to 240 kg BW [31]. Preruminant calves (90 male Holstein-Friesian × Dutch-Friesian crossbreeds) were fed a wide range of protein intakes (milk proteins only) at each of two protein-free energy intake levels.

Extra protein-free energy intake mainly results in extra fat deposition, but also increases protein deposition rate, even at low protein intakes [31]. This is in agreement with a study in ruminating steers receiving an abomasal infusion of amino acids and energy-yielding nutrients [42]. An increased protein intake results in extra fat and protein deposition [31], which corresponds with results in young preruminant calves (40–70 kg BW) [30]. Briefly, the results showed the absence of clearly distinguishable protein and energy dependent phases for protein deposition in calves, unlike the linear-plateau concept which is used for pigs. In addition, the efficiency of utilization of extra dietary protein was found to be very low: for every 100 g
increase in protein intake, protein deposition increased only with about 30 g [31]. It was concluded that the response of preruminant calves to increased nutrient intakes clearly differs from that of pigs, and that the lack of response of nitrogen balance in the amino acid requirement studies [36, 37] was not primarily caused by a limiting dietary energy supply.

4. AMINO ACID IMBALANCE

The possibility of a limiting indispensable amino acid causing both the low efficiency of nitrogen utilization in the slaughter trials [31] and the lack of response in the amino acid requirement studies [36, 37] still exists.

Gerrits et al. [43] presented the amino acid profiles in different body fractions and in the whole body of preruminant calves in the range of 80 to 240 kg BW as affected by protein and energy intake. For the detection of a possible dietary amino acid imbalance, they hypothesized that the marginal efficiency of utilization of at least one indispensable amino acid would be substantially higher than the reported marginal efficiency of 30% for total protein [43]. The marginal efficiencies found, however, were very low and within the rather narrow range of 11 to 29% for all indispensable amino acids [43]. Amongst the conditionally dispensable amino acids, arginine showed an efficiency of utilization of about 90%. The quantity of arginine ingested exceeded the quantity of arginine retained [43]. When compared with weaned pigs, which require at least 40% of the arginine requirement to be supplied by the diet, it seemed therefore unlikely that arginine was limiting protein deposition [43]. However, more recent studies suggest that arginine metabolism in neonatal, milk-fed animals may differ from that in weaned animals [44–46] (see Sect. 6.2), but it is not known if arginine supplementation affects protein deposition in heavy preruminant calves. The efficiency of utilization of cysteine was also high, on average 74%, while the efficiency of methionine was only about 27%. Utilization of methionine for the production of cysteine cannot be excluded, but the relative increase in cysteine intake with increasing protein intake was low compared with other amino acids, which may have complicated the estimation of a marginal efficiency of cysteine [43]. In conclusion, not one of the indispensable amino acids limited the rate of protein deposition. This means that other reasons are responsible for the low efficiency of protein utilization in preruminant calves.

5. AMINO ACIDS FOR AMMONIA DETOXIFICATION

A temporary high portal flux of ammonia potentially stimulates amino acid utilization for ureagenesis [47]. In ureagenesis, mitochondrial ammonia and cytosolic aspartate are precursors for the ornithine cycle. Amino acids can be used as predominant N-donors for aspartate, which incurs a penalty on amino acids available for protein synthesis [48]. Degradation of amino acids to provide precursors for the ornithine cycle could (partly) explain an inefficient utilization of amino acids for protein gain. This was shown in short-term (2–3 h) studies in sheep [49] and dairy cows [50], although other studies indicate that ammonia-N may provide more than the theoretically expected 50% of the N-atoms in urea [51–53].

In preruminant calves, feeding diets based on either skimmed milk protein or a mixture of soy and wheat gluten (50/50) results in high portal ammonia fluxes, representing 9 to 19% of the dietary nitrogen intake (or 6 and 13 g ammonia-N·d−1) respectively [54]. Nutrient absorption and consequently portal nutrient and ammonia fluxes in preruminant calves are peak-wise [55], which indicates that high fluxes of ammonia are to be processed by the liver within a relatively short time-span. The ammonia may originate from intestinal amino acid deamination, but also from protein fermentation in the gastrointestinal tract. To quantify the contribution of amino
acids to the ammonia detoxification process, Gerrits et al. [56, 57] infused ammonia, as NH₄HCO₃, in the colon of preruminant calves with an average BW of 165 kg, at each of two protein intake levels (58 and 85 g N·d⁻¹; n = 12 for each treatment), and measured the increase of nitrogen losses compared with the infusion of NaHCO₃. Infusion rates were 10 g ammonia-N per day. Also, a tracer dose of ¹⁵NH₄Cl was infused to estimate the kinetics and recovery of the infusate by analysis of ¹⁵N enrichment in urine and faeces. Faeces and urine were separately and quantitatively collected during a 6-d balance period after an adaptation period to the dietary protein intake level (17 d) and to infusion into the colon (7 d). Calculated from the total nitrogen excretion, on average only 75% (variation between animals: 0–180%) of the infused ammonia-N was recovered in urine and no interaction with protein intake was found (Tab. I). Calculated from the tracer infusion, on average 51% (variation between animals: 36–63%) of the infused ¹⁵NH₄Cl was recovered in urine. This implies that infused ammonia was only partially recovered in urine (from tracer calculations), and that the low net recovery of ammonia-N (collected up to 48 h after the end of infusion) could indicate a metabolic role of ammonia. The net recovery of infused ammonia-N was expected to exceed 100%. This would indicate extra amino acid catabolism. The low net recovery suggests, however, that no additional amino acids were catabolized to provide aspartate for the ornithine cycle. This could be due to the flexibility of the enzyme systems involved, i.e. glutamate dehydrogenase and carbamoyl-phosphate synthase I/ornithine transcarbamylase, to supply aspartate and citrulline for urea synthesis respectively. In conclusion, it is unlikely that ureagenesis itself contributes to the inefficiency of nitrogen utilization in preruminant calves.

6. ALTERNATIVE MECHANISMS

From the work described above, it was concluded that marginal efficiencies of 30–35% are normal for preruminant calves

<table>
<thead>
<tr>
<th></th>
<th>Main effects¹</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>SEM</td>
<td>P-value</td>
</tr>
<tr>
<td>Fecal N-output, g N·d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake</td>
<td>6.8</td>
<td>7.6</td>
<td>0.38</td>
<td>0.15</td>
</tr>
<tr>
<td>Ammonia infusion</td>
<td>6.9</td>
<td>7.4</td>
<td>0.23</td>
<td>0.18</td>
</tr>
<tr>
<td>Recovery ¹⁵N in feces, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake</td>
<td>6.2</td>
<td>8.4</td>
<td>0.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Urinary N-output, g N·d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake</td>
<td>27.1</td>
<td>42.5</td>
<td>0.95</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ammonia infusion</td>
<td>31.1</td>
<td>38.6</td>
<td>0.78</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Recovery ¹⁵N in urine, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake</td>
<td>48.5</td>
<td>53.9</td>
<td>1.97</td>
<td>0.07</td>
</tr>
</tbody>
</table>

¹Two-way interactions were not significant (P > 0.05).

Table I. Least squares means of fecal and urinary nitrogen output and recovery of ¹⁵N in preruminant calves at two levels of protein intake (58 vs. 83 g N·d⁻¹) and at two levels of ammonia infusion in the colon (0 vs. 10 g N·d⁻¹) with ¹⁵NH₄Cl added as a tracer to the ammonia infusion. Data from [56] (n = 12 for each treatment).
Protein utilization in preruminant calves

> 100 kg BW, using milk proteins as the sole protein source in the diet. Following a reductionist approach, it was shown that neither a protein-energy imbalance, an imbalanced amino acid profile nor the ureagenesis are responsible for the low marginal efficiency in heavy preruminant calves. Alternative hypotheses are presented and discussed below. Although individual mechanisms are described, the reason for a low efficiency of protein utilization in heavy preruminant calves may be multi-factorial in nature. Interactions between factors may be important to explain the decreased protein utilization. In order to identify the main effects and for the sake of simplicity, the mechanisms are discussed separately.

6.1. Fermentation of milk in the rumen

The estimation of the marginal efficiency of protein utilization may be complicated by ruminal drinking, certainly depending on the feeding method, but potentially also on feeding level and nutrient composition. Several studies describe the etiology and pathology of ruminal drinking [58–60], but studies reporting quantitative measurements of milk leakage into the rumen are scarce. Guilhermet et al. [61] and Wise et al. [62] used preruminant calves equipped with a rumen canula to estimate leakage of milk. Generally, both studies showed a large variation in the amount of milk recovered in the rumen between individual calves, which averaged ~ 7% [61] and 20% [62] of the milk ingested. Leakage considerably increases with age and is higher when animals are drinking from a bucket (~ 40%) than when sucking a nipple (< 1%) [61]. Tadeu dos Santos et al. [63], not mentioning the feeding method, reported 3% of spillage into the rumen in calves with a good appetite and 57% in calves with a reduced appetite. At our research station, Van Leeuwen [64] collected duodenal chyme after an oral dose of synthetic amino acids in milk and found a recovery of 84% in the duodenum. When amino acids are dissolved in water, only 10% is recovered in the duodenum [65], illustrating a poor closure of the oesophageal groove after drinking of water. Since the quantity of milk appearing in the rumen, and nitrogen losses during (protein) fermentation are unknown, it is difficult to estimate the quantitative impact of ruminal drinking on the estimated marginal efficiency of protein utilization. There are no clear indications that increasing protein intake increases ruminal drinking, and therefore has a significant impact on marginal protein efficiency. It is, however, clear that ruminal drinking can be quantitatively important, and an important source of between-animal variation.

6.2. Preferential utilization by particular tissues

Following digestion and absorption, amino acids are available for protein synthesis or oxidation. High rates of protein turnover of specific tissues, like the gut, liver, pancreas and kidney, could have affected the marginal efficiency of single amino acids as calculated by Gerrits et al. [43]. The contribution of tissues with a high turnover rate may be higher than can be expected based on their contribution to empty body protein, because high rates of protein turnover may be associated with increased oxidative losses [66]. Also, a specific preference of particular tissues to utilize indispensable amino acids can lead to an imbalance of amino acids at the site of deposition. The digestive tract, for example, can metabolize dietary indispensable amino acids in monogastrics [67] and ruminants [68]. We performed a small-scale study in which portal amino acid fluxes were measured in four preruminant calves (161 kg BW) fed clotting and non-clotting protein sources [54, 55]. The calves were surgically fitted with catheters in the portal vein, the carotid artery and the mesenteric vein and were assigned to a diet (20% crude protein) containing either skimmed milk protein or soy and wheat gluten. In a crossover design with two collection periods of two days each, portal and arterial blood
samples were taken after feeding of 1040 g milk replacer. The portal availability, as percentage of intake, of the indispensable amino acids was not affected by protein source ($P > 0.05$). Average values and comparable values for other farm animals from the literature are presented in Table II. A flawless comparison between species is hampered by different experimental conditions in conducted studies. The highly negative portal cysteine flux in the dairy cow (Tab. II), for example, was likely due to a methionine deficiency in that study. It does, however, provide some interesting leads. Firstly, the relatively high recovery of dietary indispensable amino acids in the portal vein of the milk-fed calf suggests that there is less amino acid sequestration in the portal drained viscera in preruminant calves than in the other species shown. This is presumably affected by the relatively high feeding level in the calf study. In pigs and beef steers, increasing the feeding level does not proportionally increase the irreversible loss rate over the portal drained viscera, which implies that portal amino acid recoveries increase with feed intake [78, 79]. Secondly, the absence of one poorly recovered indispensable amino acid in milk-fed calves indicates that the amino acid balance is not drastically disturbed by passing the portal drained viscera. Finally, portal arginine availability is high in milk-fed animals, both in calves and pigs. A major contribution of total arginine supply has to originate from endogenous synthesis in milk-fed animals, because milk supplies only about 60% of the arginine an animal needs for maximal growth [46, 80].

Table II. Net portal fluxes of amino acids as percentage of amino acid intake (milk-fed calf and milk-fed pig) or apparent ileal digestible amino acids (pig, dairy cow and sheep) for several farm animals.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Milk-fed calf</th>
<th>Milk-fed pig</th>
<th>Pig</th>
<th>Dairy cow</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>86</td>
<td>50</td>
<td>64</td>
<td>43</td>
<td>72</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>ND</td>
<td>ND</td>
<td>92</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Methionine</td>
<td>86</td>
<td>59</td>
<td>80</td>
<td>67</td>
<td>85</td>
</tr>
<tr>
<td>Cysteine</td>
<td>ND</td>
<td>17</td>
<td>93</td>
<td>–162</td>
<td>–3</td>
</tr>
<tr>
<td>Valine</td>
<td>89</td>
<td>67</td>
<td>74</td>
<td>51</td>
<td>68</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>85</td>
<td>74</td>
<td>83</td>
<td>62</td>
<td>75</td>
</tr>
<tr>
<td>Leucine</td>
<td>80</td>
<td>66</td>
<td>75</td>
<td>62</td>
<td>70</td>
</tr>
<tr>
<td>Lysine</td>
<td>91</td>
<td>52</td>
<td>87</td>
<td>55</td>
<td>80</td>
</tr>
<tr>
<td>Histidine</td>
<td>93</td>
<td>70</td>
<td>103</td>
<td>95</td>
<td>96</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>96</td>
<td>57</td>
<td>83</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>98</td>
<td>132</td>
<td>88</td>
<td>ND</td>
<td>97</td>
</tr>
<tr>
<td>Arginine</td>
<td>150</td>
<td>142</td>
<td>102</td>
<td>63</td>
<td>89</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>50</td>
<td>28</td>
<td>48</td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td>Serine</td>
<td>101</td>
<td>71</td>
<td>98</td>
<td>75</td>
<td>117</td>
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<tr>
<td>Glutamic acid</td>
<td>3</td>
<td>–2</td>
<td>–132</td>
<td>9</td>
<td>–51</td>
</tr>
<tr>
<td>Glycine</td>
<td>87</td>
<td>61</td>
<td>75</td>
<td>42</td>
<td>134</td>
</tr>
<tr>
<td>Alanine</td>
<td>180</td>
<td>190</td>
<td>215</td>
<td>80</td>
<td>107</td>
</tr>
<tr>
<td>Proline</td>
<td>65</td>
<td>87</td>
<td>48</td>
<td>9</td>
<td>95</td>
</tr>
</tbody>
</table>

citrulline production, which is converted to arginine by the kidney [81]. Studies on arginine metabolism have mainly been performed in milk-fed pigs during the first 21 d of life [46]. Arginine supplementation has been shown to increase growth in young calves [44, 45], but the mechanisms involved have not yet been studied. From this point of view, the high marginal efficiencies for arginine in heavy preruminant calves (97% for 80–160 kg BW; 89% for 160–240 kg BW) [43] may have some biological significance. Conversely, the low marginal efficiency of proline (~ 35%), which is a precursor for endogenous arginine synthesis, did not suggest any limitation of arginine availability. Arginine kinetics have not been studied in preruminant calves and quantification of arginine synthesis and the interaction with the ornithine cycle activity as well as identification of the tissue(s) involved would be interesting.

The liver is the second tissue which can be studied individually. Although several studies report hepatic nutrient fluxes in preruminant calves [82–85], the fate of individual amino acids is not described and amino acid catabolism by the liver can not be quantified. In conclusion, the few data available do not directly suggest preferential utilization of amino acids by specific tissues in preruminant calves.

6.3. Recycling of urea nitrogen

Recycling of urea by microbes in the gastrointestinal tract of dairy cows (67% of urea synthesis) and sheep (61% of urea synthesis) contributes to a large extent to the nitrogen supply in ruminant animals and can even exceed the apparent digestible nitrogen intake [86]. In milk-fed calves, this partial reuse of urea is expected to be negligible due to the absence of non-starch polysaccharides provoking microbial activity in the gastrointestinal tract. The recovery of an intravenous pulse dose of $^{13}$C-urea was about 80% in 48 h urine of milk-fed calves [56], which indicates that urea recycling is substantially lower than in ruminants [86]. Provision of roughage (viz. potential carbon precursors) will definitely increase urea recycling and therefore the utilization of digestible protein for growth, provided that ruminal drinking is not promoted by the provision of roughage.

6.4. Utilization of amino acids for gluconeogenesis

Donkin and colleagues [87–89] have intensively studied gluconeogenesis in hepatocyte monolayers of milk-fed calves as affected by insulin and glucagon. They showed that the hepatic capacity for gluconeogenesis from lactate in milk-fed calves is much higher than in ruminant calves. Also, Ortigues et al. [85] suggested that the Cori cycle can be of greater importance in preruminant than in ruminants. For propionate, the gluconeogenic capacity was shown to be at least as high as in ruminant calves [88]. This substantial capacity for gluconeogenesis in preruminant calves seems to be redundant, because large amounts of monosaccharides are supplied by the diet. On the contrary, ruminating animals have an obligate need for gluconeogenesis, and it has been suggested that gluconeogenesis from amino acids is quantitatively important, even if the dietary glucose supply is abundant [90]. If genetically predisposed, preruminants potentially catabolize amino acids for gluconeogenesis, causing a low marginal efficiency of protein utilization.

In vivo quantification of gluconeogenesis and especially the contribution of different precursors (e.g. lactate, glycerol and amino acids), in preruminant calves is lacking. Therefore, despite the considerable gluconeogenic capacity and enzyme activity in milk-fed calves, the quantity of amino acids used for gluconeogenesis is not known.

6.5. Decreased insulin sensitivity

Heavy preruminant calves often develop a certain level of insulin resistance, indicated by postprandial hyperglycemia, hyperinsulinemia and glucosuria [91, 92]. The
etiology of insulin resistance in preruminant calves is likely to be multi-factorial, as reviewed by Blum and Hammon [93]. A high level of circulating insulin down-regulates its own receptors, which may result in altered cellular glucose and amino acid transport and compromised protein synthesis. Feeding large amounts of milk (especially lactose) to ontogenically ruminant animals, may contribute to the development of insulin resistance [93, 92]. In addition, increasing protein intake decreased insulin resistance, as indicated by glucosuria and high plasma concentrations of glucose and insulin, in preruminant calves [94]. Mechanistic explanations for the effect of protein intake on insulin resistance are not yet clarified. If insulin resistance coincides with reduced protein utilization, this effect will increase with increasing protein intake, thus contributing to a low marginal efficiency of protein utilization.

The consequences of insulin resistance for the utilization of amino acids in preruminant calves are, however, unknown. The effects of non-insulin-dependent diabetes on protein utilization in human subjects are generally absent [95, 96], although Gougeon et al. [97] reported increased rates of protein breakdown and a more negative nitrogen balance in diabetic subjects. The low feeding level (non-growing man) and post-absorptive measurements complicate the extrapolation of these results to quickly growing milk-fed calves. Moreover, endocrine regulation of protein metabolism in the heavy preruminant calf is not unambiguous, because the responsiveness to insulin is apparently different in ruminants and non-ruminants [98–100]. Insufficient insight in the regulation of protein metabolism in preruminant calves raises the question if insulin resistance contributes to the low marginal efficiency of protein utilization.

6.6. Post-absorptive nutrient asynchrony

Although all macronutrients are provided simultaneously to preruminant calves, an asynchronous post-absorptive availability of individual nutrients may occur. Separation of amino acid and glucose availability in time, for example, can be expected when skimmed milk protein is fed to calves. Casein (~80% of milk protein) clots in the calf abomasum and leads to a slow release of protein into the intestinal tract [5, 83, 101], while lactose does not have clotting abilities and is absorbed relatively quickly compared with dietary protein [5, 102] (Fig. 3). An asynchronous availability of glucose and amino acids within a day was shown to substantially decrease protein utilization in the adult man [reviewed by 103] and growing rats [104].

Apart from an asynchrony between individual nutrients, also an asynchrony between total nutrient supply and total nutrient requirements within a day may occur. Preruminant calves are usually fed twice daily, resulting in two peaks of metabolically available nutrients, while in ruminants, these patterns are absent because of eating patterns and gradual passage of feed through the rumen compartment. It can be hypothesized that in milk-fed calves, the efficiency of protein utilization can to some extent be the consequence of a temporary excessive amino acid availability. In man, for example, it has been shown that protein retention increases with a slower digestion rate of dietary protein [105, 106]. The peak-wise glucose availability, however, requires flexibility of metabolic pathways to clear glucose from the systemic circulation. A disturbed glucose metabolism may also indirectly affect protein utilization (see previous section).

Finally, asynchrony occurs when individual amino acids are not available at the same moment. In calf milk replacers, vegetable protein sources (mainly soy, wheat, potato) have partly replaced skimmed milk protein during the past decades, with subsequent addition of crystalline amino acids to reach an ideal amino acid pattern for growth. Supplementation of the limiting amino acids as crystalline amino acids to
the feed compensates this limitation and improves growth performance. Absorption kinetics of the supplemented free amino acids and the protein-bound amino acids, however, differ [107, 108] and may lead to an amino acid imbalance at the site of deposition.

Skimmed milk protein and respectively synthetic amino acids [36, 37] or caseinates [31] were used as protein sources in the studies which demonstrated the low efficiency of protein utilization in preruminant calves. Therefore, the contribution of each type of nutrient asynchrony to the low marginal efficiency may have varied between studies. In general, interpretation of studies about nutrient asynchrony is complicated, since the three types of nutrient asynchrony
are often confounded. The use of a slowly digestible protein source [105], for example, results in a more gradual supply of amino acids during the day, but also in an increased asynchrony between amino acid and glucose absorption. Separate effects of different types of nutrient asynchrony can counteract, as shown by the interaction between protein source and feeding frequency on amino acid oxidation in pigs [109].

7. CONCLUSIONS AND IMPLICATIONS

It was determined that the utilization of extra dietary protein for protein gain is lower in preruminant calves than in several other species of farm animals. Especially preruminant calves above 100 kg BW showed a low marginal efficiency of protein utilization (< 30%). The inefficient utilization could not be explained by either a protein to energy imbalance or an imbalanced amino acid profile in the diet. Also the utilization of amino acids for ammonia detoxification did not contribute to the low efficiency. Alternative mechanisms result in either a reduced post-absorptive supply of amino acids or any indispensable amino acid (e.g. fermentation of milk or preferential utilization by tissues), or a reduced utilization of the post-absorptive available amino acids (e.g. insulin resistance or nutrient asynchrony). Nonetheless, the possibility of multiple factors being involved should not be excluded.

The low efficiency of protein utilization illustrates that there is a large potential for improvement of the efficiency of growth in heavy preruminant calves. More insight in the amino acid metabolism of preruminant calves is needed to decrease nitrogen losses. Furthermore, mechanisms of amino acid utilization in preruminant calves could also be relevant for ruminants in which similar studies are hampered by rumen fermentation processes.
Protein utilization in preruminant calves


Protein utilization in preruminant calves 135


[75] Van der Meulen J, Bakker JGM, Smits B, De Visser H. Effect of source of starch on net por-tal flux of glucose, lactate, volatile fatty acids


