Effect of Protein Supply on Hepatic Synthesis of Plasma and Constitutive Proteins in Lactating Dairy Cows

G. Raggio,* G. E. Lobley,† R. Berthiaume,‡ D. Pellerin,* G. Allard,* P. Dubreuil,§ and H. Lapierre‡

*Department of Animal Science, Université Laval, Ste-Foy, Quebec, Canada, G1K 7P4
†Rowett Research Institute, Aberdeen, UK, AB21 9SB
‡Dairy and Swine Research and Development Center, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada, J1M 1Z3
§Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Quebec, Canada, J2S 7C6

ABSTRACT

The effects of metabolizable protein (MP) supply on the synthesis of plasma total proteins and albumin, as well as total hepatic protein synthesis, were determined in 6 multicatheterized lactating Holstein cows. Three TMR formulated to supply the same amount of energy but different amounts of MP, 1,922 (low), 2,264 (medium), and 2,517 g of MP/d (high), were fed every 2 h according to a double 3 × 3 Latin square design. For the low and high MP treatments, the cows were continuously infused with [2H5]Phe (d5-Phe) into a jugular vein for 8 h (1.3 mmol/h) on d 21 of each period. Concentration and isotopic enrichment of d5-Phe were measured for free plasma Phe, plasma total proteins, and albumin on hourly samples collected between 3 and 8 h. Low MP decreased the plasma albumin concentration (32.3 vs. 33.7 ± 0.11 g/L) but the plasma total protein concentration was unchanged (74.1 vs. 75.6 ± 1.13 g/L). Incorporation of d5-Phe over time into both plasma total proteins and albumin was linear (R² > 0.98). Neither fractional nor absolute synthesis rates of plasma total proteins (6.8 vs. 6.5 ± 0.65%/d; 168 vs. 154 ± 19.9 g/d) or albumin (3.4 vs. 3.4 ± 0.10%/d; 36.3 vs. 36.5 ± 1.11 g/d) were affected by the MP supply. Net hepatic removal of Phe was lower with the low-MP diet (−12.3 vs. −20.2 ± 1.98 mmol/h). As a result, net hepatic Phe removal used for total export protein synthesis (17.9 vs. 11.1 ± 1.83%) and albumin synthesis (4.6 vs. 2.9 ± 0.54%) tended to be greater at low MP. These results suggest that hepatic synthesis of plasma proteins, including albumin, is maintained in lactating dairy cows when the protein supply is reduced.

Key words: liver, albumin, protein, dairy cow

INTRODUCTION

The liver is the major site of removal of certain AA in both ruminants and nonruminants (Rérat et al., 1992; Lobley and Lapierre, 2003; Hanigan, 2005). In dairy cows, for example, approximately 45% of net portal appearance of total AA is extracted by the liver (Lapierre et al., 2005). However, this composite value masks the considerable variation among AA, with fractional removals of net portal appearance of essential AA varying between 0 (for the branched-chain AA) and 50% (Phe). Despite the fact that the liver is the principal site of ureagenesis in the body (Lapierre and Lobley, 2001), these hepatic extractions do not encompass only catabolism. Instead, part of the net removal can also be used for synthesis of export proteins (Jahoor et al., 1996; Connell et al., 1997) plus any net accretion of liver protein during growth (Burrin et al., 1990).

The different hepatic export proteins play a variety of roles, including maintenance of vascular osmotic pressure (e.g., albumin), coagulation (fibrinogen), immunity (C-reactive protein), and antiphagocytic mechanisms (α1-antitrypsin, α1-antichymotrypsin, α2-macroglobulin). These functions are vital to the metabolic integrity of the animal, and synthesis of export proteins is therefore maintained even under conditions of nutritional deprivation, including low protein intakes and fasting in sheep and humans (Hunter et al., 1995; Connell et al., 1997; Barber et al., 2000). This is important because the contribution of export proteins to total hepatic synthesis can be considerable; for example, albumin accounted for 18% of total liver protein synthesis in fattening lambs (Connell et al., 1997). Such synthesis may have implications for the AA supply beyond the liver and for support of anabolic processes. For example, some export proteins contain proportionally high amounts of certain AA, and their synthesis may then limit the posthepatic supply of these AA in free form to support peripheral anabolism, particularly in the case of Phe and Cys as suggested by Reeds et al. (1992). Of course, these export proteins may provide an anabolic source of AA to peripheral tissues, many of which have the ability to degrade albumin (Eskild et al., 1989; Maxwell et al., 1990). Protein sources, including those in blood, can provide short-term reservoirs of AA and have the advantage of not inducing regulatory mecha-
nisms that would accompany the supply of similar quantities of the free AA.

Therefore, the objectives of this study were 1) to quantify, in lactating dairy cows, the rate of synthesis of plasma protein synthesis; 2) to estimate the maximum proportion of net hepatic removal of Phe the liver could use to synthesize export proteins; 3) to establish the maximum proportion of hepatic protein synthesis directed toward export proteins vs. constitutive proteins; and 4) to determine how these parameters are affected by protein supply.

MATERIALS AND METHODS

Animals, Treatments, and Sampling

Six multiparous Holstein cows, averaging 656 ± 60 kg of BW and 96 ± 8 DIM at the beginning of the study were used in a double 3 × 3 Latin square design balanced for residual effects with 21-d experimental periods. All cows were surgically implanted with catheters in the portal vein, in 1 hepatic and 2 mesenteric veins, and in a mesenteric artery. In addition, the right carotid artery was surgically raised to a subcutaneous position (see Raggio et al., 2004). Three diets were balanced to provide the same amount of energy (36.4 Mcal NEL/d) but with increasing amounts of MP, averaging 1,922 (low), 2,264 (medium), and 2,517 g (high) of MP/d (NRC, 2001). Diets were as detailed previously (Raggio et al., 2004) and were offered from automated feeders (Ankom, Fairport, NY) in equal quantities every 2 h, except for 1 kg of long hay that was offered once a day. The cows had free access to water and were housed in a tie-stall barn, lit from 0600 to 2200 h. Cows were milked twice daily (0600 and 1800 h) and the yield was recorded at each milking.

On d 18, 19, or 20 of each experimental period, measurements were made of splanchic plasma flows by downstream dilution of p-aminohippurate and of mammary plasma flow using the Fick principle, as reported previously (Raggio et al., 2004). On these days, hematocrit also was determined by microcentrifugation in capillary tubes. At 0600 h on the last day of each period (d 21), cattle on the low- and high-MP diets received an 8-h (1.3 mmol/h) infusion of [5H5]Phe (d5-Phe; Cambridge Isotope Laboratories, Andover, MA; 99 atom %), preceded by a priming dose (1.3 mmol). Infusions of d5-Phe were limited to the low and high treatments because of the cost of the tracer. Blood samples were collected simultaneously every hour from the artery plus portal and hepatic veins starting 3 h after initiation of the infusion (n = 6). Blood samples from the mammary vein were obtained by venipuncture every other hour (n = 3). Two blood samples were taken from each catheter prior to the start of the infusion for determination of natural abundance. Immediately after collection in plastic syringes, blood was transferred into 10-mL heparinized Vacutainers (sodium heparin spray coated, 150 USP units per tube; Becton, Dickinson and Company, Franklin Lakes, NJ) and kept on ice.

The experimental protocol was approved by the Committee for Animal Care of the Lennoxville Research Center and conducted according to the guidelines of the Canadian Council on Animal Care (1993).

Laboratory Analyses

Plasma was obtained immediately following blood sampling by centrifugation at 1,000 × g for 15 min at 4°C. Plasma Phe concentrations were measured by the gravimetric isotope dilution method (Calder et al., 1999). On the day of sampling, 0.2 g of an internal standard (226 μM of [1-13C]Phe, 99 atom %; C/D/N isotopes, Montreal, Quebec, Canada) was added to 1 g of plasma and the mixture was stored at −20°C for later analysis. At the time of analysis, these samples, as well as plasma taken for isotopic enrichment (IE) analysis, were thawed, mixed, deproteinized with sulfosalicylic acid (380 g/L), and desalted on AG-50 H+ resin (Sigma-Aldrich, St. Louis, MO). The freeze-dried eluate was derivatized with N-(tert-butylidemethylyl)-N-methyltrifluoroacetamide:acetronile (1:1) to form the N-(tert-butylidemethyl) AA derivative (Calder and Smith, 1988). The Phe IE, as molar percent excess (mpe) above preinfusion values as either plasma free Phe or that present in protein, were quantified by GC-MS as either m/z ions 321, 322 (to determine concentrations on samples with the internal standard, [1-13C]Phe) or as m/z ions 336, 341 (to determine the IE of plasma free d5-Phe; GC-MS, Hewlett Packard model GC6890-MS5973; Agilent Technologies, Wilmington, DE).

In addition, determination of the enrichment of d5-Phe incorporated into plasma total proteins and albumin was determined as described by Connell et al. (1997) as m/z ions 336, 341 (GC-MS Voyager; Thermquest, Wythenshawe, Lancashire, UK). Determination of the concentration of plasma total proteins and of albumin was performed on a clinical analyzer (Kone Instruments Corporation, Espoo, Finland) calibrated with the appropriate standard supplied by the manufacturer (Thermo Electron Systems, Waltham, MA).

Calculations and Statistical Analyses

During the infusion of d5-Phe, the temporal increase in IE of d5-Phe incorporated into plasma total proteins and albumin was plotted against time (between 3 and 8 h) to determine linear regressions, as described previously (Connell et al., 1997). The slope was used in
conjunction with the appropriate precursor pool (taken as the IE of free d5-Phe in the hepatic vein at plateau, i.e., from 3 to 8 h of infusion; see Connell et al., 1997) to calculate the fractional rate of protein synthesis of both plasma total proteins and albumin.

Daily fractional rates (FSR) of plasma total proteins (PTP) and albumin (Alb) were calculated as follows:

\[
FSR \text{ (\%/d)} = \text{slope} \times \text{IE of PTP or Alb vs. time (h)} / \text{IE}_{\text{H}},
\]

where IE was expressed as the mpe. Half-life (d) was estimated from \(\ln(2)/\text{FSR}(100)\).

The absolute rates of PTP and Alb synthesis (ASR) were calculated as

\[
\text{ASR} \text{ (g/d)} = \text{FSR} \times [\text{PTP or Alb} \times \text{PV}],
\]

where \([\text{PTP or Alb}]\) refers to the plasma concentrations of plasma total proteins or albumin (g/L), respectively, and \(\text{PV}\) refers to the plasma volume (L), assumed to average 49.9 mL/kg of BW (Girard et al., 1989). To estimate the requirement of Phe for the absolute synthesis rate of plasma total proteins or albumin, we assumed that plasma total proteins contain 5% Phe (Connell et al., 1997) and that albumin contains 6% Phe (Peters, 1985).

Net fluxes of Phe across the portal-drained viscera (PDV), liver, and splanchnic tissues (TSP = PDV + liver) as well as mammary gland were calculated for each cow as the product of the average of the venous-arterial concentration difference and the plasma flow, estimated on the day prior to the d5-Phe infusion (see Raggio et al., 2004). A negative flux meant utilization or removal, whereas a positive flux indicated net production or release.

The whole body (WB) Phe irreversible loss rate (ILR; mmol/h) was calculated as follows:

\[
\text{WB-ILR} = (\text{IE}_{\text{inf}} / \text{IE}_{\text{A}} - 1) \times \text{Inf},
\]

where \(\text{IE}_{\text{inf}}\) and \(\text{IE}_{\text{A}}\) are the IE of the infusate and the mean IE of d5-Phe in arterial plasma free Phe, and \(\text{Inf}\) is the infusion rate (mmol/h) of d5-Phe.

Phenylalanine ILR (mmol/h) through the liver was calculated as follows:

\[
\text{Hepatic-ILR} = -[(\text{IE}_{\text{PTP}} - \text{PFP}) \times \text{PF}] + [\text{IE}_{\text{Phe-P}} \times \text{PFP}] / \text{IE}_{\text{H}},
\]

where the subscript indicates the site of plasma collection (A for artery, P for portal vein, H for hepatic vein) and IE_{\text{Phe}}, [Phe], and PF represent, respectively, the IE (mpe) and concentration (mM) of plasma free Phe and the plasma flow (L/h) in the corresponding vessel.

Because the cows were in midlactation, changes in liver protein mass were assumed to be zero; therefore, hepatic Phe oxidation (mmol/h) was estimated to be the difference between net Phe liver removal minus Phe used for plasma total protein synthesis. Phenylalanine used in the liver for protein synthesis was then calculated as

\[
\text{hepatic protein synthesis} = \text{hepatic ILR} - \text{hepatic oxidation}.
\]

Phenylalanine used for the synthesis of constitutive protein in the liver was calculated as the difference between that used for total hepatic protein synthesis and that used for the synthesis of plasma total proteins.

One cow did not have a patent hepatic catheter and her portal catheter was also misplaced. Therefore, data for this cow were discarded from all analyses. In addition, one cow suffered from severe mastitis during her last period (on high MP), and this cow period was also excluded. Therefore, \(n = 5\) for low MP and \(n = 4\) for high MP. Data were analyzed statistically using the MIXED procedure of SAS (SAS Institute, 2004). Because this study was part of the double 3 Latin square reported earlier (Raggio et al., 2004), but without the intermediate treatment and with the missing cows, data were analyzed according to a randomized block design, with treatment and cow as fixed factors. Treatment differences were considered significant if \(P \leq 0.05\) and as a tendency with \(0.05 < P \leq 0.10\).

**RESULTS**

The increase in MP supply resulted in a 15% improvement in milk protein yield (848 vs. 980 g/d, \(P = 0.05\)) as reported previously (Raggio et al., 2004). For both plasma total proteins and albumin, there was a linear (\(R^2 \geq 0.98\)) incorporation of d5-Phe at between 3 and 8 h of the d5-Phe infusion (Figure 1). Synthesis rates, both fractional and absolute, of plasma total proteins and albumin plus their concentrations are presented in Table 1. The albumin concentration increased by 4% (\(P = 0.02\)) with the high-MP diet. The hematocrit values did not change between treatments (29.6 vs. 29.7 ± 0.33%, \(P = 0.80\)).

Concentrations and IE of plasma free Phe are presented in Table 2. The concentration of Phe increased (\(P \leq 0.07\)) in all blood vessels with the high-MP diet, whereas the IE of Phe decreased (\(P \leq 0.03\)). Phenylalanine net flux across the PDV tended to increase (23.5
vs. 32.7 mmol/h; \( P = 0.06 \) with the high-MP diet (Table 3). This was accompanied by increased \( P = 0.05 \) net hepatic Phe removal with, as a result, no change \( P = 0.72 \) in net supply beyond the total splanchnic tissues. The maximum proportion of hepatic Phe removal used for export of plasma total protein synthesis \( (17.9 \text{ vs. } 11.1 \pm 1.83\%; \ P = 0.07) \) and for albumin synthesis \( (4.6 \text{ vs. } 2.9 \pm 0.54\%; \ P = 0.09) \) tended to decrease at the higher MP supply. There was only a numerical increase \( (P = 0.18) \) in mammary gland net uptake of Phe between

![Figure 1](image1.png)

Figure 1. Average of the isotopic enrichment (IE, mpe = molar percent excess) of Phe in plasma total proteins and albumin during a continuous infusion of \(^{[2H_5]}\text{Phe}\) at a low (A) or high (B) MP supply.

Table 1. Effect of supply of metabolizable protein (MP) on concentrations and synthesis rates of plasma total proteins and albumin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proteins</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma concentration, g/L</td>
<td>Total proteins</td>
<td>Low MP</td>
<td>74.1</td>
<td>75.6</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>High MP</td>
<td>32.3</td>
<td>33.7</td>
<td>0.11</td>
</tr>
<tr>
<td>Fractional synthesis rate, %/d</td>
<td>Total proteins</td>
<td>Low MP</td>
<td>6.8</td>
<td>6.5</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>High MP</td>
<td>3.4</td>
<td>3.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Absolute synthesis rate, g/d</td>
<td>Total proteins</td>
<td>Low MP</td>
<td>168</td>
<td>154</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>High MP</td>
<td>36.3</td>
<td>36.5</td>
<td>1.11</td>
</tr>
</tbody>
</table>

\(^{1}\) Least squares means are presented with the highest pooled SEM; \( n = 5 \) for low MP, and \( n = 4 \) for high MP.
Table 2. Effect of supply of MP on the Phe concentration and isotopic enrichment in plasma samples during an 8-h infusion of \([^{2}H_{5}]\text{Phe}\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site</th>
<th>Low MP</th>
<th>High MP</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe Concentration, (\mu M)</td>
<td>Arterial</td>
<td>59.7</td>
<td>71.6</td>
<td>2.61</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Portal</td>
<td>77.7</td>
<td>98.2</td>
<td>2.50</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Hepatic</td>
<td>66.6</td>
<td>79.3</td>
<td>2.07</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Mammary</td>
<td>38.3</td>
<td>50.1</td>
<td>2.64</td>
<td>0.07</td>
</tr>
<tr>
<td>Isotopic enrichment, mpe(^{2})</td>
<td>Arterial</td>
<td>3.5</td>
<td>2.9</td>
<td>0.09</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Portal</td>
<td>2.3</td>
<td>2.0</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Hepatic</td>
<td>2.3</td>
<td>1.9</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Mammary</td>
<td>3.5</td>
<td>2.8</td>
<td>0.11</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\(^{1}\)Least squares means are presented with pooled SEM (given for \(n = 4\)).

\(^{2}\)mpe = Mole percent excess.

Table 3. Effect of supply of MP on net transfers of Phe (mmol/h)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Low MP</th>
<th>High MP</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal-drained viscera</td>
<td>23.5</td>
<td>32.7</td>
<td>2.34</td>
<td>0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>−12.3</td>
<td>−20.2</td>
<td>1.98</td>
<td>0.05</td>
</tr>
<tr>
<td>Total splanchnic tissue</td>
<td>11.2</td>
<td>12.5</td>
<td>2.64</td>
<td>0.72</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>−11.3</td>
<td>−12.9</td>
<td>0.56</td>
<td>0.18</td>
</tr>
<tr>
<td>Milk</td>
<td>10.5</td>
<td>12.1</td>
<td>0.45</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(^{1}\)Least squares means are presented with pooled SEM (given for \(n = 4\)); a positive number indicates a release and a negative number indicates an uptake across the tissue.

Phenylalanine kinetics are presented in Table 4. The high-MP diet increased \((P < 0.01)\) the whole body ILR, whereas the Phe hepatic ILR increased by 51% \((P = 0.04)\). This latter effect was due to a combination of increased hepatic oxidation \((P = 0.05)\) and hepatic protein synthesis \((P = 0.04)\). The synthesis of constitutive proteins increased with the high-MP diet \((P = 0.03)\), with a tendency for these to represent a higher proportion of total hepatic protein synthesis (83.3 vs. 88.4 ± 1.39%; \(P = 0.06)\).

DISCUSSION

Our objectives were to examine whether the MP supply altered 1) the synthesis of plasma proteins, including albumin, which are mainly synthesized by the liver; 2) the proportion of net hepatic Phe removal not catabolized but used to support the synthesis of export proteins (which could support milk protein production); and 3) the proportion of hepatic protein synthesis directed toward export proteins vs. constitutive proteins. However, interpretation of the results requires considering the limitations of the techniques used. Because multicatheterized animals were used, liver biopsies were not performed and the IE of the hepatic precursor pool for protein synthesis was therefore assumed to be similar to the IE of plasma free d5-Phe in the hepatic vein. In sheep, this has been shown to be a valid assumption for estimating the synthesis of export proteins (Connell et al., 1997), formed on the rough endoplasmic reticulum in close contact with the extracellular pools. For constitutive protein synthesis, however, the precursor IE is probably lower and close to that in the hepatic cytosol (Fern and Garlick, 1976; Smith and Sun, 1995; Connell et al., 1997). Because this intracellular IE could not be estimated in the current study, the values of the constitutive protein synthesis are underestimated. Based on data from fed sheep, this underestimate would approximate to 25% (Connell et al., 1997). Furthermore, plasma total proteins involve a mixture derived from liver (including albumin, fibrinogen, and 60 to 80% of plasma globulins) and other sources (e.g., the γ-globulins are synthesized in lymphoid tissues and other cells of the reticuloendothelial system; Ridley and Field, 1963). Therefore, assuming that plasma total proteins are all hepatic-derived will yield an overestimation of liver activity. Nonetheless, provided that the 2 MP supplies do not influence either the relationship between intracellular:vascular IE or the proportion of export proteins derived from liver, then the relative responses to diet can be properly considered.

The absolute synthesis rates of both plasma total proteins and albumin were unaltered by the change in MP supply. Studies in other species have reported albumin synthesis to be sensitive to nutrient supply, with decreases in both 3-d starved sheep (Connell et al., 1997) and overnight-fasted humans (Hunter et al., 1995). In addition, a low protein supply also decreases albumin synthesis in humans (Barber et al., 2000) and pigs (Jahoor et al., 1999). The reported experiments exerted moderate to severe nutrient deprivations, whereas in the current study, the lowest MP supply...
Table 4. Effect of supply of MP on Phe kinetics (mmol/h)1

<table>
<thead>
<tr>
<th>Item</th>
<th>Low MP</th>
<th>High MP</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body ILR2</td>
<td>35.4</td>
<td>42.9</td>
<td>0.87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hepatic ILR</td>
<td>23.3</td>
<td>35.3</td>
<td>2.66</td>
<td>0.04</td>
</tr>
<tr>
<td>Hepatic oxidation</td>
<td>10.2</td>
<td>18.2</td>
<td>1.89</td>
<td>0.05</td>
</tr>
<tr>
<td>Hepatic total protein synthesis</td>
<td>13.0</td>
<td>17.1</td>
<td>0.92</td>
<td>0.04</td>
</tr>
<tr>
<td>Export plasma protein synthesis</td>
<td>2.1</td>
<td>2.0</td>
<td>0.25</td>
<td>0.64</td>
</tr>
<tr>
<td>Hepatic constitutive protein synthesis</td>
<td>10.9</td>
<td>15.1</td>
<td>0.81</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein synthesis, constitutive:total</td>
<td>0.83</td>
<td>0.88</td>
<td>0.014</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1Least squares means are presented with pooled SEM (given for n = 4).
2ILR = Irreversible loss rate.

still supported over 30 kg of milk/d. Therefore, the lack of response in export protein synthesis in these lactating cows may reflect the relatively mild nature of the nutrient differences or, alternatively, may indicate that demands on the lactating cow require maintenance of plasma protein synthesis to ensure biological integrity and function. Indeed, in comparative terms, plasma total protein synthesis, expressed on a metabolic BW basis, is slightly higher in the lactating dairy cow than in the nonlactating sheep (1.24 vs. 1.02 g/kg of BW0.75, current study and Connell et al., 1997). A similar difference was observed for albumin (0.28 vs. 0.22 g/kg of BW0.75). This latter difference may reflect the need for export albumin (and other plasma proteins) in the milk, which averages 14 g of albumin (based on 0.4 g of albumin/kg of milk), representing 38% of albumin synthesis.

Hepatic export protein synthesis did not change even though the net portal absorption of Phe was decreased on the low-MP diet, as was the hepatic removal. Therefore, at the lower MP supply, the proportion of the Phe removed by the liver used for export protein synthesis increased, whereas Phe oxidation decreased by 33%. Interestingly, although export protein synthesis was not affected by protein supply, the estimate of constitutive protein synthesis was reduced markedly (39%, P = 0.03) at the lower MP. This meant that a substantial change occurred in the proportion of total liver protein synthesis represented by export proteins between the high and low MP supply (17 vs. 12%). These proportions were lower than in nonlactating sheep (38 to 51%; Connell et al., 1997) and reflect, for the dairy cow, a higher estimated fractional synthesis rate for constitutive proteins, 0.49 to 0.68/d [based on 788 and 1,089 g of constitutive protein/d for low and high MP, respectively; a liver size of 1.47% BW (Reynolds et al., 2004), and 16.7% CP in the liver (C. Girard, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada; personal communication)] compared with 0.12/d in sheep (Connell et al., 1997). In practice, the total plasma synthesis includes proteins produced from nonhepatic tissues, and the contributions from these may differ between species and physiological states. These proportional differences between species may also be magnified by the higher sensitivity of hepatic constitutive protein synthesis to protein intake in the lactating cows (+39%) compared with no responses to total intake in the nonlactating sheep.

As a result of the increased MP supply, the net portal appearance of Phe increased, and this led to a 14 to 30% increase in plasma Phe concentrations and consequent elevated net Phe hepatic removal, as observed in other studies (Bach et al., 2000; Blouin et al., 2002). Net hepatic removal represented 53 and 61% of net portal absorption at a low and high MP intake, respectively, with Phe usually one of the AA extracted in the greatest proportion relative to portal absorption in lactating cows (Hanigan, 2005; Lapiere et al., 2005). However, the fraction of removal relative to total inflow to the liver (i.e., total flow from the portal vein plus the hepatic artery) did not differ between MP supplies, representing 10 vs. 13% of total inflow for low vs. high MP, respectively. Hanigan (2005) and Lapiere et al. (2005) have previously suggested that the fractional hepatic extraction of AA relative to total inflow is relatively constant in lactating cows. Postsplanchnic free Phe supply matched both uptake and output (milk) by the mammary gland, as observed in a number of recent reports (see Lobley and Lapiere, 2003; Lapiere et al., 2005). Such findings negate the need to invoke plasma protein as a potential source of AA, at least for milk synthesis.

Based on the present findings, the hypothesis that synthesis of hepatic export proteins may limit free Phe supply postliver for anabolism (Reeds et al., 1992) is not supported by our data in the healthy, lactating cow. Furthermore, although export proteins can provide a source of AA to tissues, as reported for skin, muscle, and liver (Eskild et al., 1989; Maxwell et al., 1990), this does not seem to be necessary to support mammary gland metabolism, as assessed from the close quantitative agreement between supply and utilization for milk
protein secretion. Whether AA from export proteins are utilized in the dairy cow for anabolic purposes by nonhepatic tissues or enter catabolic pathways beyond the liver (e.g., degradation within the intestinal tract; Poppi et al., 1986) cannot be identified from the current study. If they do provide an anabolic component, this would supply, at a maximum, an additional 16 to 20% above that available as free Phe.

CONCLUSIONS

In healthy, midlactation dairy cows, synthesis of either plasma total proteins or albumin is not altered by MP supply. Their constant synthesis, across a 30% change in MP intake, suggests that their metabolic priority was maintained even though milk protein yield was altered by 15%. Although a larger proportion of Phe extracted by the liver was used to support export protein synthesis at a low MP supply, this was offset by lower hepatic oxidation, so the amount of free Phe available to support milk protein production remained unchanged.

ACKNOWLEDGMENTS

The authors gratefully thank the staff of the Dairy and Swine R&D Centre for taking care of the animals; M. Léonard, S. Provencier, and J. Renaud for their dedicated technical support; and S. Méthot for statistical analyses. Grateful thanks are extended to F. Boutchakourt for isolation of the plasma albumin and total proteins and to S. Anderson for the mass spectrometric analyses of the protein-bound enrichments. The authors also wish to acknowledge H.J. Baker & Bro. Inc. (Stamford, CT) for supplying the Pro-Lak bypass protein supplement and the financial support of the Action concertée Fonds FCAR–Novalait–MAPAQ, the National Science and Engineering Research Council of Canada, the Rowett Research Institute through a grant from the Scottish Executive Environmental and Rural Affairs Department and Agriculture and Agri-Food Canada (Contribution number 902).

REFERENCES


