Protein Synthesis in Jejunum and Liver of Neonatal Calves Fed Vitamin A and Lactoferrin

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ABSTRACT

Rates of protein synthesis (PS) and turnover are more rapid during the neonatal period than during any other stage of postnatal life. Vitamin A and lactoferrin (Lf) can stimulate PS in neonates. However, newborn calves are vitamin A deficient and have a low Lf status, but plasma vitamin A and Lf levels increase rapidly after ingestion of colostrum. Neonatal calves (n = 6 per group) were fed colostrum or a milk-based formula without or with vitamin A, Lf, or vitamin A plus Lf to study PS in the jejunum and liver. L-[13C]Valine was intravenously administered to determine isotopic enrichment of free (nonprotein-bound) Val (APFree) in the protein precursor pool, atom percentage excess (APE) of protein-bound Val, fractional protein synthesis rate (FSR) in the jejunum and liver, and isotopic enrichment of Val in plasma (APEPla) and in the CO2 of exhaled air (APEEx). The APE, APFree, and FSR in the jejunum and liver did not differ significantly among groups. The APEEx increased, whereas APEPla decreased over time, but there were no group differences. Correlations were calculated between FSRjej and histomorphometrical and histochemical data of the jejunum, and between FSRLiv and blood metabolites. There were negative correlations between FSRLiv and plasma albumin concentrations and between FSRjej and the ratio of villus height:crypt depth, and there was a positive correlation between FSRjej and small intestinal cell proliferation in crypts. Hence, there were no effects of vitamin A and Lf and no interactions between vitamin A and Lf on intestinal and hepatic PS. However, FSRjej was correlated with histomorphometrical traits of the jejunum and FSRLiv was correlated with plasma albumin concentrations.

Key words: neonatal calf, lactoferrin, protein synthesis, vitamin A

INTRODUCTION

The rate of protein synthesis (PS) is more important during the neonatal period than during any other stage of postnatal life (Davis and Reeds, 1998b) and is maximally stimulated after feeding (O’Connor et al., 2004). In the gastrointestinal tract (GIT) and in the liver, the PS rate is much greater than in other organs (Stoll et al., 1999). After birth, the route of nutrition shifts from largely parenteral to enteral, and rapid protein accretion in the GIT of neonates is needed for GIT development for efficient digestion and absorption of nutrients (Stoll et al., 2000). Davis et al. (1998a) showed that in the liver of neonates, the stimulation of PS by feeding primarily depends on the AA supply. In addition, there are reports of a PS-stimulating role of vitamin A and lactoferrin (Lf) in neonates (DeLuca et al., 1969; Burrin et al., 1996).

Lactoferrin is a multifunctional glycoprotein of the transferrin family. It is known to act as a growth factor by stimulating mucosal growth of the small intestine (Roblee et al., 2003) and to increase hepatic PS in newborn pigs (Burrin et al., 1996). Newborn calves have low circulating amounts of Lf, but after ingestion of colostrum, which contains high amounts of Lf, plasma Lf concentrations increase rapidly (Muri et al., 2005). Therefore, calves that receive supplemental Lf are able to absorb more nutrients from their feed and are more efficient in converting feed into BW gain (Roblee et al., 2003).

Vitamin A is well known to be an essential food component that is involved in vision, maintenance of healthy epithelium, growth, bone formation, reproduction, and immune function and also to be important in calves (Konermann and Abou El Fadle, 1966; Franklin et al., 1998). The PS in the GIT is depressed during
vitamin A deficiency, indicating that the vitamin is involved in PS (De Luca et al., 1969). Calves are born with very low liver reserves and low plasma concentrations of vitamin A (Blum et al., 1997; Franklin et al., 1998; Zanker et al., 2000), but after ingestion of colostrum—which normally contains high amounts of vitamin A—plasma vitamin A concentrations increase rapidly (Blum et al., 1997; Zanker et al., 2000).

To the best of our knowledge, studies on the influence of vitamin A and Lf on PS in the jejunum and liver of neonatal calves have not yet been published. Based on these premises, we tested the hypothesis that ingested vitamin A and Lf influence jejunal and hepatic PS in neonatal calves and that there are interactions between vitamin A and Lf. Calves were fed colostrum or a formula without or with vitamin A, Lf, or vitamin A plus Lf. By using a nonradioactive tracer of Val-L-[13C]Val, isotopic enrichments of 13C in the plasma, exhaled air, jejenum, and liver were measured.

MATERIALS AND METHODS

Experimental Design

Experimental Procedures. Experimental procedures were approved by the Cantonal Committee for the Permission of Animal Experimentation (Granges-Paccot, Canton of Freiburg, Switzerland) and were under the supervision of the Swiss Federal Veterinary Office (Liebefeld-Bern).

Animals. Thirty male calves were selected from a group of 35 calves in the frame of a larger study on the effects of vitamin A and Lf (Muri et al., 2005; Schottstedt et al., 2005). Because the calves were not allowed to be used for human consumption and because the study was expensive and time consuming, the number of experimental animals was kept as low as possible. The calves were born after pregnancies of normal duration (285 ± 1 d) between February and May of 2003 at the Experimental Station (Posieux, Switzerland) and at neighboring farms. After birth, the calves were immediately separated from their dams and kept on straw in individual boxes.

Experimental Groups, Feeds, and Feeding. Five differently fed groups were created, each consisting of 6 calves. Calves of group CO (2 Holstein-Friesian and 4 Simmental × Red Holstein) were fed pooled colostrum obtained from milkings 1, 3, and 5 (d 1, d 2, and d 3 of lactation, respectively) on the first 3 d of life, and the colostrum of milking 5 was fed on d 4. Group CO was considered the control group and was fed colostrum that contained vitamin A (49, 61, and 76 µmol/kg of DM in milkings 1, 3, and 5, respectively) and Lf (2,030, 1,110, and 560 mg/kg of DM in milkings 1, 3, and 5, respectively).

Calves of group FO (1 Red Holstein calf and 5 Simmental × Red Holstein) were fed a milk-based formula that was not supplemented with vitamin A and Lf. Calves of group FLf (3 Holstein-Friesian and 3 Simmental × Red Holstein), group FA (2 Holstein-Friesian and 4 Simmental × Red Holstein), and group FALf (2 Holstein-Friesian and 4 Simmental × Red Holstein) were fed the same formula in the same amounts as group FO but the formula was supplemented with Lf (group FLf), vitamin A (group FA), or vitamin A plus Lf (group FALf).

Vitamin A was added during the production of the different formulas, which were kept at room temperature and protected from light. Measured retinol and Lf values in the formulas and colostrum are shown in Table 1. In group FLf, 3,850, 1,990, 660, and 660 mg of Lf/kg of DM was added to the formulas that were fed on d 1, 2, 3, and 4, respectively. In group FA, 351, 402, 490, and 490 µmol of vitamin A/kg of DM was added to the formulas that were fed on d 1 (day of birth), 2, 3, and 4, respectively. In group FALf, 351, 402, 490, and 490 µmol of vitamin A/kg of DM and 3,850, 1,990, 660, and 660 mg of Lf/kg of DM was added to the formulas that were fed on d 1, 2, 3, and 4, respectively. The Lf (no. 10116702; DMV International, Veghel, The Netherlands) was dissolved in double-distilled water (1 g/20 mL) for better handling, and was mixed into the formula immediately before feeding.

Colostrum was collected from cows at the Experimental Station (Posieux, Switzerland). Cows were milked twice daily and the colostrum of milkings 1, 3, and 5 after parturition was stored separately in plastic bottles at −20°C. Individual pools of milkings 1, 3, and 5 were prepared at the beginning of the study and then stored in plastic bottles at −20°C until used. Before feeding, colostrum was warmed to 40°C and then fed immediately.

Formulas were produced by UFA AG (Sursee, Switzerland) and consisted of calcium caseinate (Emmi Milch AG, Lucerne, Switzerland), lactalbumin (Emmi Milch AG), and a vitamin and mineral premix that contained vitamin A only if fed to groups FA and FALf (Provimi S.A., Cossonay-Gare, Switzerland). Formula powders were dissolved in water, and fat was added during the mixing procedure (49.7% saturated fatty acids, 39.0% unsaturated fatty acids, 6.7% polyunsaturated fatty acids, 2.1% trans fatty acids, and 2.5% water; # 763, Nutriswiss AG, Lyss, Switzerland). The added fat contained no measurable amounts of vitamin A. Lecithin, as emulsifier (Emulsifier LO-1; UFA AG), was added and made up 3% of the total fat. Formulas were planned to contain amounts of nutrients comparable to colostrum. Compositions and concentrations of
### Table 1. Composition of fed colostrum and formulas

<table>
<thead>
<tr>
<th>Parameter expressed per kg fresh weight (in parentheses, per kg of DM)</th>
<th>Colostrum&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Formula&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Colostrum</th>
<th>Formula</th>
<th>Colostrum</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, g/kg</td>
<td>251</td>
<td>248</td>
<td>164</td>
<td>139</td>
<td>144</td>
<td>127</td>
</tr>
<tr>
<td>Gross energy, MJ/kg (MJ/kg of DM)</td>
<td>6.2 (24.7)</td>
<td>6.2 (25.0)</td>
<td>4 (24.0)</td>
<td>3.3 (23.7)</td>
<td>3.4 (23.8)</td>
<td>3 (23.6)</td>
</tr>
<tr>
<td>CP, g/kg (g/kg of DM)</td>
<td>159 (634)</td>
<td>152 (613)</td>
<td>77 (470)</td>
<td>60 (431)</td>
<td>58 (403)</td>
<td>48 (378)</td>
</tr>
<tr>
<td>Crude fat, g/kg (g/kg of DM)</td>
<td>53 (211)</td>
<td>59 (238)</td>
<td>39 (238)</td>
<td>29 (209)</td>
<td>36 (250)</td>
<td>30 (236)</td>
</tr>
<tr>
<td>Nitrogen free extract, g/kg (g/kg of DM)</td>
<td>27 (108)</td>
<td>26 (105)</td>
<td>23 (232)</td>
<td>45 (324)</td>
<td>41 (285)</td>
<td>45 (354)</td>
</tr>
<tr>
<td>Ash, g/kg (g/kg of DM)</td>
<td>12 (47)</td>
<td>11 (44)</td>
<td>10 (61)</td>
<td>5 (36)</td>
<td>9 (63)</td>
<td>4 (32)</td>
</tr>
<tr>
<td>Vitamin A, µmol/kg (µmol/kg of DM)</td>
<td></td>
<td></td>
<td>12 (49)</td>
<td>10 (61)</td>
<td>11 (76)</td>
<td></td>
</tr>
<tr>
<td>Formula vitamin A-free&lt;sup&gt;3&lt;/sup&gt;</td>
<td>—</td>
<td>NM</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td>ND</td>
</tr>
<tr>
<td>Lactoferrin, mg/kg (mg/kg of DM)</td>
<td>511 (2,030)</td>
<td>182 (1,110)</td>
<td>80 (560)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula lactoferrin-free</td>
<td>—</td>
<td>4 (16)</td>
<td>2 (14)</td>
<td>1 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula lactoferrin-added</td>
<td>—</td>
<td>955 (3,850)</td>
<td>277 (1,890)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Colostrum fed on d 1; measurements on d 2 and d 3 and 4 of life were derived from pooled milkings 1, 3 and 5. Vitamin E concentrations in colostrum milkings 1, 3, and 5 were 9, 6 and 7 g/kg, respectively.

2Formulas (per kg of DM and fed on d 1, 2, and 3 and 4 of life, respectively) were composed of calcium caseinate (97, 238, and 334 g, respectively), lactalbumin (603, 250, and 64 g, respectively), fat (180, 180, and 220, respectively), lactose (64, 275, and 322 g, respectively), and a mineral premix (56, 57, and 59 g, respectively). Fat per kilogram contained 497, 390, 67, 21, and 25 g saturated, unsaturated, polyunsaturated, and trans fatty acids, and water, respectively. The mineral premix (per kilogram) contained calcium (186 g), magnesium (224 g), sodium (31 g), phosphorus (92 g), chlorine (48 g), iron (12 g), manganese (8.1 mg), copper (1.6 mg), zinc (7.8 mg), iodine (0.03 mg), cobalt (0.02 mg), and selenium (0.02 mg). Vitamin E concentrations in formulas fed on d 1, 2, and 3 and 4 were 294, 138, and 97 g/kg, respectively.

3NM = not measurable; ND = not determined.

### Table 2. Total ingested feed for the 4 d of experimentation

<table>
<thead>
<tr>
<th>Component</th>
<th>Colostrum, milking 1</th>
<th>Formula, d 1</th>
<th>Colostrum, milking 3</th>
<th>Formula, d 2</th>
<th>Colostrum, milking 5</th>
<th>Formula, d 3 and 4</th>
<th>Total ingested feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, g/kg of BW</td>
<td>15</td>
<td>14.8</td>
<td>13</td>
<td>11.1</td>
<td>14.4</td>
<td>12.7</td>
<td>56.8</td>
</tr>
<tr>
<td>Gross energy, MJ/kg of BW</td>
<td>0.37</td>
<td>0.37</td>
<td>0.31</td>
<td>0.31</td>
<td>0.34</td>
<td>0.30</td>
<td>1.37</td>
</tr>
<tr>
<td>Crude protein, g/kg of BW</td>
<td>9.55</td>
<td>9.12</td>
<td>6.16</td>
<td>4.80</td>
<td>5.80</td>
<td>4.80</td>
<td>27.3</td>
</tr>
<tr>
<td>Crude fat, g/kg of BW</td>
<td>3.18</td>
<td>6.54</td>
<td>3.12</td>
<td>2.32</td>
<td>3.60</td>
<td>2.99</td>
<td>13.5</td>
</tr>
<tr>
<td>Nitrogen-free extract, g/kg of BW</td>
<td>1.63</td>
<td>1.56</td>
<td>3.04</td>
<td>3.80</td>
<td>4.10</td>
<td>4.49</td>
<td>12.9</td>
</tr>
<tr>
<td>Ash, g/kg of BW</td>
<td>0.7</td>
<td>0.65</td>
<td>0.80</td>
<td>0.40</td>
<td>0.90</td>
<td>0.41</td>
<td>3.32</td>
</tr>
<tr>
<td>Vitamin A, µmol/kg of BW</td>
<td>0.75</td>
<td>0.80</td>
<td>1.09</td>
<td></td>
<td>3.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Formula vitamin A-free&lt;sup&gt;1&lt;/sup&gt;</td>
<td>NM</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Formula vitamin A-added</td>
<td>5.22</td>
<td>4.47</td>
<td>6.22</td>
<td></td>
<td>22.14</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lactoferrin, mg/kg of BW</td>
<td>30.6</td>
<td>8.06</td>
<td>61.3</td>
<td></td>
<td>6.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Formula lactoferrin-free</td>
<td>0.24</td>
<td>0.16</td>
<td>0.10</td>
<td></td>
<td>0.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Formula lactoferrin-added</td>
<td>57.3</td>
<td>22.1</td>
<td>8.4</td>
<td></td>
<td>96.2</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1NM = not measured; ND = not determined.
egg-derived immunoglobulins that contained high antibody titers against rotavirus and pathogenic *Escherichia coli* type K99 (Globigen 88; Lohmann Animal Health, Cuxhaven, Germany). Amounts fed per meal on d 1, 2, 3, and 4 were 5, 4, 3, and 2 g, respectively. On d 2, 3, and 4, calves received a subcutaneous injection of antibiotics (25 mg of enrofloxacin per 10 kg of BW; Baytril 5%; Bayer AG, Leverkusen, Germany). Navels were disinfected with Betadine (Mundipharma, Basel, Switzerland) shortly after birth.

**Health Status.** The following clinical traits were evaluated daily: rectal temperature, heart rate, respiratory rate, behavior, nasal discharge, eye discharge, respiratory sounds, cough, appetite, and fecal consistency. The navel and joints were checked daily. The BW was determined preprandially on d 1, 4, and 5.

**Protein Synthesis Measurements.** On d 5, an infusion of L-[1-13C]Val was administered according to the “flooding dose” method (Garlick et al., 1989) as described by Sève et al. (1993). The solution was prepared 12 to 14 h before starting the infusion by mixing 20% of L-[1-13C]Val (Cambridge Isotope Laboratories, Andover, MA) with 80% of unlabeled L-Val (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to give 19.6 mol percent excess of final enrichment and by diluting with distilled water to give a final concentration of 0.15 mol/L. The mixture was slightly heated to 37°C for better dissolution, cooled, and stored at 4°C until used. Before injection, the solution was sterilized through a 0.22-μm mesh filter (Millipore AG, Volketswil, Switzerland) and heated to body temperature. Approximately 18 min before euthanasia, the infusion was started by using a Perpex rotation pump (Witec AG, Littau, Switzerland). Five calves from each group (randomly assigned) received an injection of the mixture, whereas one calf from each group (randomly assigned) received an unlabeled L-Val infusion (Sigma-Aldrich Chemie GmbH). The solutions were injected into the calves via a jugular catheter at a dose of 1.05 mmol/kg of BW during approximately 6 min. The time when half of the injection was given was considered to be time zero of labeling (t₀ = zero). After injection, the catheter was rinsed with a solution of physiological saline. After the last blood and breath samples were taken, the calves were euthanized with 80 mg of pentobarbital/kg of BW (Eutha 77; Essex Animal Health, Friesoythe, Germany).

**Blood, Air, and Tissue Sampling**

**Blood Plasma.** Four blood samples were drawn (1 before and 3 after the infusion). The first sample was taken before the infusion had started (t₀ – 3 min). The second blood sample was taken at the end of the infusion (t₀ + 3 min). Samples 3 and 4 were taken after 7 min relatively to time zero (t₀ + 7 min) and after 14 min relatively to time zero (t₀ + 14 min), respectively. Samples were kept sun protected in tubes containing dithioerythritol (1.8 g/L of blood) on crushed ice until centrifuged (1,000 × g, 4°C, 20 min). Supernatants were aliquoted and stored at −20°C until analysis.

**Exhaled Air.** Exhalation air was collected before and after the infusion with a self-constructed respiratory mask, at −3, +3, +7, and +14 min (relative to t₀) at the same time the blood samples were taken. The air was stored at room temperature in evacuated tubes (LABCO Ltd., Geneva, Switzerland) until analysis.

**Tissues.** The gut was removed within 5 min after euthanasia and transferred into ice-cold PBS. Pieces (10 to 20 g) of jejunum were collected in plastic boxes and placed in liquid nitrogen. Livers were immediately removed, weighed, and samples of lobus caudatus (1 to 2 g) were snap-frozen in liquid nitrogen. Both tissues were stored at −80°C until analysis.

**Laboratory Analyses**

**Formulas and Colostrum.** At the Swiss Federal Research Station (Agroscope Liebefeld-Posieux, Switzerland), samples of colostrum pools from milkings 1, 3, and 5 and formulas for d 1, 2, and 3 were lyophilized to determine DM, CP (Kjeldahl method), crude fat (direct extraction), and crude ash (after combustion at 550°C) using standard procedures. Contents of nitrogen-free extract and gross energy were calculated. Vitamin A in formula powders supplemented with vitamin A (for d 1, 2, and 3) and without supplemented vitamin A (for d 1) was analyzed at the Swiss Federal Research Station ALP, Liebefeld (Buetikofer and Andersson, 1978; Muri et al., 2005). Contents of vitamin A in colostrum were analyzed at the Swiss Federal Research Station ALP, Liebefeld (Buetikofer and Bøsset, 1994). Concentrations of Lf in the formulas and colostrum were measured with an ELISA kit (Bethyl, Montgomery, TX) that was specific for bovine Lf (Muri et al., 2005). Colostrum and formula samples were centrifuged at 14,000 × g for 15 min, followed by removing the phase between the fat layer and precipitate, which was subjected to analysis with ELISA.

**Blood Metabolites.** Plasma Lf concentrations were measured with an ELISA kit (Bethyl) using an antibody that was specific for bovine Lf. Plasma retinol concentrations were measured by an HPLC method with diode-array detection adapted for calf plasma analysis at the University of Ghent (Muri et al., 2005).

**Exhaled Air.** The exhaled air was collected in evacuated tubes (LABCO Ltd.) and was measured by infrared...
mass spectrometry (Roboprep CN-Tracermass; Europa Scientific, Crewe, UK) within days after sampling.

**Free L-[13C]Val in Plasma and Tissues Supernatants.** The atom percent (AP) excess of free L-[13C]Val in plasma (AP\textsubscript{PL}\text{free}) and tissue supernatants (AP\textsubscript{JejFree}, AP\textsubscript{LivFree}) were measured by gas chromatography–mass spectrometry using the following procedure. Pieces of liver and jejunum (1 to 2 g) were homogenized in 5 mL of ice-cold 0.2 mol/L perchloric acid with a homogenizer (B. Braun Melsungen AG, Melsungen, Germany). Plasma samples (500 µL) were deproteinized with an equal volume of sulfosalicylic acid (15%). The acid-soluble fractions containing free AA of plasma and tissue supernatants were separated from the protein precipitate by centrifugation (1,800 × g, 4°C, 20 min). The AA were isolated by passing the solutions through a cation-exchange resin (AG-50W-X8, 100-200 mesh, H⁺ form; BioRad Laboratories AG, Glattbrugg, Switzerland). These columns were conditioned before use by washing them 3 times with 1 M HCl and distilled water. The elution of AA was carried out with 2 mL of 2 M ammonium hydroxide buffer. After evaporation, the AA were dried and resuspended in 200 µL of 0.1 M HCl. The determination of Val enrichment was performed by gas chromatography–mass spectrometry with an HP 5971 organic mass spectrometer quadrupole coupled to an HP 5890 gas chromatograph (Agilent Technologies Switzerland AG, Geneva, Switzerland). Electron-impact ionization and a selected ion monitoring mode of mass-to-charge-ratios of 288 and 289 were used to quantify the isotopic ratio of the tertiary butyl-dimethylsilyl derivative of free Val. The AA derivatives were separated using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies Switzerland AG). Injections (1 µL) were made in the splitless mode. The injector temperature was 280°C and the oven temperature was programmed starting at 160°C for 5 min, and then increasing to 270°C by 15°C/min. Standard curves were constructed for each sequence of analysis by dissolving weighted amounts of 20% L-[1-13C]Val and 80% unlabeled L-Val in distilled water to make serial standard dilutions.

**Bound L-[13C]Val in Tissues.** Samples of liver and jejunum (1 to 2 g) were homogenized in 5 mL of ice-cold 0.2 mol/L perchloric acid with a homogenizer (B. Braun Melsungen AG) and then centrifuged (1,800 × g, 4°C, 20 min). The supernatants, containing the protein precursor pool, were used to determine nonprotein or free L-[13C]Val enrichment (see above). The pellet, constituting the protein-bound or protein-incorporated pool of L-[13C]Val, was homogenized 3 more times with 10 mL of 0.2 mol/L ice-cold perchloric acid to remove any free AA and was centrifuged each time (1,800 × g, 4°C, 20 min). The pellet was then rehomogenized with 10 mL of 0.3 M NaOH solution and shaken for 1 h at 37°C. The proteins were precipitated once more with 2.5 mL of 2 mol/L ice-cold perchloric acid and then centrifuged (1,800 × g, 4°C, 20 min). The pellet was resuspended twice with 10 mL of 0.2 mol/L ice-cold perchloric acid and hydrolyzed in 40 mL of 12 M HCl at 110°C for 48 h in 5 sealed tubes. The HCl was removed by evaporation (Rotavapor R-114; Buchi Laborotechnik AG, Flawil, Switzerland) at 80°C and 4 kPa. The hydrolysate was collected in 1,000 µL of 0.05 mol/L sodium acetate (pH 5.7, +3% tetrahydrofuran) and then centrifuged (1,800 × g, 4°C, 10 min). For the measurement of isotopic enrichment, protein-bound tissue Val was first purified by HPLC (Gilson AG, Mettmensen, Switerland). A 100-µL quantity of sample was injected on the column (Silice Uptisphere HDO; 120 5 µm; Interchem, Laubescher Laboratories, Miccourt, Switzerland) under the following conditions: 20°C; flow rate of 1 mL/min; 13,000 kPa. The mobile phase consisted of sodium acetate (0.05 mol/L, pH 5.7, +3% tetrahydrofuran) and methanol (+0.05% tetrahydrofuran). The eluate was collected after approximately 4 min. The sample was dried for 90 min under vacuum (Speedvac; Eglyrolyt AG, Oberwil, Switzerland) and for 20 min under a stream of nitrogen. The tube was filled with nitrogen for approximately 30 s to ensure that no carboxyl was left in the tube. The carboxyl group of Val was released by the ninhydrin reaction. The 13C enrichment of the CO₂ produced (ratio 13CO₂/12CO₂) was measured by an isotope ratio mass spectrometer (Roboprep CN-Tracermass; Europa Scientific), which was equipped with a triple collector to simultaneously measure intensities of the mass-to-charge ratio equal to 44, 45, and 46. Standard curves were prepared by dissolving weighted amounts of 20% L-[1-13C]Val and 80% unlabeled L-Val in distilled water and then measured into each analyzed batch to make serial standard dilutions.

**Histological Analyses.** Methods used for histomorphological evaluations and to measure cell proliferation based on the incorporation of 5-bromo-2'-deoxyuridine (BrdU) have recently been described (Schottstedt et al., 2005). Data on these traits, from the same animals as in the present study, were taken from Schottstedt et al. (2005).

**Calculations, Data Presentation, and Statistics**

The atomic weights of stable isotopes are slightly heavier because of additional neutrons in their nucleus. Therefore, they can be separated according to mass. They are present in small quantities (natural abundance ≤1%), but in constant proportions relative to the
Figure 1. Atom percent excess of L-[13C]Val in the blood plasma of calves fed formula (FO), formula plus lactoferrin (FLf), formula plus vitamin A (FA), formula plus vitamin A and lactoferrin (FALf), or colostrum (CO). Samples were taken on d 5 at +3, +7, and +14 min relative to midinjection time (t0 = 0). Values are means (n = 5 per group), and SEM of groups FO, FLf, FA, FALf, and CO are represented by strokes aligned at the bottom of the figure for each corresponding time and from left to right for groups FO, FLf, FA, FALf, and CO. The same data are shown on a logarithmic scale in the inset panel to demonstrate that the clearance rate of the tracer out of circulation in all 5 groups was similar.

The fractional protein synthesis rate (FSR; fraction or percentage of the tissue protein renewed per day) was determined based on the rate of incorporation of L-[1-13C]Val. Calculations of the FSR of tissue proteins require isotopic enrichment to be measured in both the precursor pool (i.e., intracellular free Val) and in the protein-bound pool. The PS was calculated as a fractional rate (Ks, %/d) from the equation described by Garlick et al. (1989): Ks = 100 × (APEjej or Liv/APJej or LivFree/t), where APEjej or Liv is the enrichment of bound L-[1-13C]Val in jejunal or liver tissue protein, APJej or LivFree is the enrichment of free L-[1-13C]Val (i.e., the precursor pool) in tissues, and t is the duration of labeling in d, calculated at slaughter, starting at midinjection time (t0 = zero).

The data were analyzed with NCSS software (2004; NCSS, Kaysville, UT) and SAS version 8.2 (SAS Inst.,...
Figure 2. Atom percent excess of $^{13}$C in the exhaled air of calves fed formula (FO), formula plus lactoferrin (FLf), formula plus vitamin A (FA), formula plus vitamin A and lactoferrin (FALf), or colostrum (CO). Samples were taken on d 5 at +3, +7, and +14 min relative to midinjection time ($t_0 = 0$). Values are means ($n = 5$ per group), and SEM of groups FO, FLf, FA, FALf, and CO are represented by strokes aligned at the bottom of the figure for each corresponding time and from left to right for groups FO, FLf, FA, FALf, and CO.

The results of interval-measured variables are presented as means ± SEM or medians and quartiles to ranges if they were clearly not normally distributed. The nonparametric Kruskal–Wallis ANOVA test on ranks was used to compare measurements between feeding groups. A generalized linear model ANOVA was used to assess differences in the means for AP, APE, FSR, and free Val of the jejunum and liver between the feeding groups. The overall level for statistical significance was set at $P < 0.05$. The association between interval-measured variables was assessed using the Spearman rank correlation coefficient.

RESULTS

Body and Liver Weights, Health Status, and Rectal Temperature

There were no significant differences among groups for birth weights (mean of all calves: $45.9 \pm 0.8$ kg), BW on d 5 (mean of all calves: $45.8 \pm 0.8$ kg), and liver weights (mean of all calves: $1.08 \pm 0.03$ kg or $2.37 \pm 0.06\%$ of BW). Calves were generally healthy, but fecal consistency was slightly watery from d 3 on in formula-fed calves. Rectal temperature increased ($P < 0.05$) similarly in all groups from d 1 to 5 (means of all calves: $38.3 \pm 0.2$ and $39.0 \pm 0.1^\circ$C, respectively).

Feeding

Colostrum-fed calves ingested on average $56.8$ g of DM, $1.4$ MJ of gross energy, $27.3$ g of CP, $13.5$ g of crude fat, $12.9$ g of nitrogen-free extract, $3.3$ g of ash, $3.7$ μmol of vitamin A, and $61$ mg of Lf/kg of BW per d (Table 2). Total feed intakes as fed were similar in the 5 groups, but during the 4-d period, calves of group CO received slightly higher amounts ($P < 0.1$) of DM, gross energy, CP, crude fat, and ash and slightly lower amounts ($P < 0.1$) of nitrogen-free extract than formula-fed calves. During the 4-d trial, formula-fed calves ingested $51.3$ g of DM, $1.2$ MJ of gross energy, $23.5$ g of CP, $11.9$ g of crude fat, $14.2$ g of nitrogen-free extract, and $1.9$ g of ash/kg of BW per d. Calves of groups FA and FALf ingested on average $22.1$ μmol of vitamin A/kg of BW per d, whereas those of groups FO and FLf did not ingest measurable amounts of vitamin A. Calves of groups FLf and FALf ingested on average $96$ mg of
Lf/kg of BW per d, whereas those of groups FO and FA ingested 0.6 mg of Lf/kg of BW per d.

Isotopic Enrichment in Plasma, Exhaled Air, and Tissues

**Plasma and Exhaled Air.** In all groups, APE\textsubscript{Pla} rapidly increased after injection of the isotope and reached maximal values when the injection was completed (t\textsubscript{0} + 3 min), and then decreased (Figure 1). The APE\textsubscript{Pla} was affected by time (P < 0.001), but was not significantly affected by group. The clearance of the tracer out of circulation was comparable in all groups, as shown in the inset graph with the logarithmic scale.

After injection of the tracer, AP\textsubscript{Ex} (not shown) and APE\textsubscript{Ex} increased (P < 0.001) up to t\textsubscript{0} + 14 min (Figure 2). In calves that were injected with unlabeled L-Val, the exhaled \textsuperscript{13}CO\textsubscript{2} remained stable, as expected (not shown).

**Tissues.** In the jejunum and liver, values of AP, APE, and AP\textsubscript{Free} (not shown) and of FSR were similar in all groups and not statistically different (Figures 3, 4).

**Correlations**

As shown in Tables 3 and 4, FSR\textsubscript{Jej} was positively correlated with the thickness of jejunal muscularis externa, jejunal crypt depth, jejunal BrdU-labeled cell number:total villus cell number ratios, and BrdU-labeled crypt cell number:total crypt cell number ratios. In contrast, FSR\textsubscript{Jej} was negatively correlated with villus height and with the villus height:crypt depth ratios. No significant correlations were found between FSR\textsubscript{Jej} and the thickness of jejunal submucosa.

The FSR\textsubscript{Liv} was positively correlated with plasma urea concentrations, was negatively correlated with total protein, and was significantly negatively correlated with plasma albumin concentrations (P < 0.05). No significant correlations were found between FSR\textsubscript{Liv} and relative and absolute liver weights.

<table>
<thead>
<tr>
<th>Trait</th>
<th>FSR\textsubscript{Liv}, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus height, (\mu m)</td>
<td>-0.25</td>
</tr>
<tr>
<td>Crypt depth, (\mu m)</td>
<td>0.38</td>
</tr>
<tr>
<td>Villus height:crypt depth</td>
<td>-0.49\textsuperscript{*}</td>
</tr>
<tr>
<td>Submucosa, (\mu m)</td>
<td>0</td>
</tr>
<tr>
<td>Muscularis externa, (\mu m)</td>
<td>0.37</td>
</tr>
<tr>
<td>BrdU-labeled crypt cell:total crypt cells (\times 100)</td>
<td>0.42\textsuperscript{*}</td>
</tr>
<tr>
<td>BrdU-labeled cell:total villus cells (\times 100)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Calves were without food for about 12 h before euthanasia on d 5. BrdU = 5-bromo-2'-deoxyuridine.

\textsuperscript{*}Significantly different (P < 0.05) from zero.
Table 4. Spearman rank coefficients of correlation between fractional protein synthesis rate (FSR) of liver, liver weights, and blood parameters of calves fed colostrum or formula without or supplemented with vitamin A, lactoferrin, or vitamin A plus lactoferrin in the first 5 d of life

<table>
<thead>
<tr>
<th>Item</th>
<th>FSR_jej, %</th>
<th>Total protein, g/L</th>
<th>Albumin, g/L</th>
<th>Urea, mmol/L</th>
<th>Liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSR, %</td>
<td>0</td>
<td>-0.36</td>
<td>-0.46*</td>
<td>0.10</td>
<td>0</td>
</tr>
</tbody>
</table>

1Calves were without food for about 12 h before euthanasia on d 5. ND = not determined.
*Significantly different (P < 0.05) from zero.

DISCUSSION

Health Status, BW, and Rectal Temperature

Calves were generally healthy. Only the slightly watery fecal consistency from d 3 on in formula-fed calves indicated a mild disturbance of GIT function. However, there were no indications of (histo)pathological intestinal changes (Schottstedt et al., 2005), and there were no differences in other health traits among the groups (Muri et al., 2005). The BW remained stable during the study period and did not differ among groups, suggesting that feed utilization in the different groups was similar. The increase in rectal temperature, observed in all groups during the experimental period, was in accordance with other studies (Hadorn et al., 1997) and may be interpreted as a sign of increased vitality and metabolic activity.

Feeding

Feeding. In accordance with previous studies (Hadorn et al., 1997), there was a decrease in DM, gross energy, CP, crude fat, and ash contents, but an increase in nitrogen-free extract content in colostrum during the first days of lactation. Feed compositions of formulas for each day were intended to be similar to those of colostrum, but actual measurements revealed that calves of group CO ingested slightly more DM, CP, crude fat, gross energy, and ash and less nitrogen-free extract, vitamin A, and Lf than formula-fed calves. However, this had no marked influence on PS in the jejunum and liver in this study. Other studies in piglets (Burrin et al., 1992) and lambs (Patureau-Mirand et al., 1990) showed that animals fed colostrum consumed more protein than those fed milk. In colostrum-fed piglets, more AA were absorbed, which were available for intestinal and hepatic PS. The calves were without food for about 14 h before euthanasia on d 5. The liver, in particular, is very sensitive to any kind of alteration in food intake (O’Connor et al., 2004) and that the dietary AA are efficiently utilized as direct precursors for protein deposition (Davis et al., 1998a).

Contents of Lf in colostrum were lower than in other studies (Tsuji et al., 1990; Blum and Hammon, 2000), and reasons for these distinctions are not known. However, differences in breed, lactation number, health status of the udder, and stage of mammary involution are known to influence the colostral Lf content (Tsuji et al., 1990). Concentrations of Lf in the formulas to which Lf was added were slightly higher than those in the corresponding colostrum.

The content of vitamin A in colostrum depends on several factors, such as β-carotene and vitamin A intake of the dam, season, day of lactation, and parity number of the dam (Konermann and Abou El Fadle, 1966; Franklin et al., 1998). In the present study, pooled colostrum contained amounts of vitamin A that were comparable with those of previous studies and its concentration declined over time, as shown by Konermann and Abou El Fadle (1966). The amount of vitamin A in the formulas was approximately 7 times higher than in colostrum and led to a marked rise of plasma vitamin A concentrations (Muri et al., 2005; Schottstedt et al., 2005).

Tracer Studies

Methodological Aspects. Two techniques of tracer administration (the flooding dose technique and the “constant infusion” technique) are used to evaluate PS. In the constant infusion technique, a labeled AA tracer is administered for several hours until, ideally, a steady state of tracer in plasma and in the intracellular pools of the body is reached (Rathmacher, 2000). This method is optimal for the measurement of slow turnover proteins. In the present experiment the flooding dose technique was used, which involves the injection of a relatively large amount of unlabeled AA together with the tracer AA to minimize differences in isotopic enrichment of the free AA in plasma and tissue compartments (Garlick et al., 1989). This technique is particularly well suited for the study of tissues with rapid protein...
turnover and associated cell renewal (Egerland et al., 1996), is as expected to be the case in neonatal animals. In contrast to the constant infusion technique, improved detection of precursor enrichment and shorter periods of measurement are additional advantages of this method (Rathmacher, 2000). Valine was chosen as a carrier of the tracer based on metabolic and physiochemical criteria. Valine has several advantages compared with other AA used in other tracer studies: 1) It is safe because even high amounts are nontoxic (Egerland et al., 1996); 2) it is relatively inexpensive (Sève et al., 1993); 3) it is the most soluble essential AA; 4) it can easily be purified by chromatography; 5) it does not stimulate insulin secretion, which is a well-known factor that increases PS (Egerland et al., 1996); and 6) the Val content in proteins is generally high (Egerland et al., 1996). Hence, relatively high PS rates can be expected, thus allowing a relatively sensitive measurement system to be established. However, the tracer influences the $K_s$ value because the PS rate depends on the incorporation rate of an AA and not on all steps that finally result in protein deposition (Egerland et al., 1996). Therefore, the synthesis rate differs depending on which AA is used.

**Isotopic Enrichment in Plasma and Exhaled Air.** The increase of $^{13}$C in exhaled air and the decrease of plasma $L$-[13C]Val over time were expected and can be explained by metabolization (exhaled air) and disappearance out of the plasma space, respectively. The time between the administration of the tracer and the sampling of the air (15 min) was too short to make a reasonable statement on exhalation data with respect to PS rate. The $^{13}$C in the exhaled air likely reflected the initial oxidation of the tracer and thus depended on the physical variables involved in CO$_2$ metabolism, time needed for passage through the whole-body bicarbonate pool before being expired, and metabolic rate.

**General Aspects of Intestinal and Hepatic Protein Synthesis of Neonatal Calves.** Rates of protein PS and breakdown are influenced by several factors, such as age, nutrition, stress, disease, hormones, and physical activity (Rathmacher, 2000). Davis et al. (1991) showed that the skeletal muscles of young suckling rats, despite their functional immaturity, are able to rapidly downregulate PS by reducing the uptake of AA, which are then redistributed for use in other organs. This adaptation may be essential to maintain the supply of AA to organs that need AA for proper functioning. All tissues are competing for AA from the plasma pool, except the GIT and liver, which receive an exogenous supply of AA from the diet (Seal and Parker, 2000). Furthermore, the rate of PS in a tissue depends on the number of ribosomes per cell, the activity of these ribosomes, and the capacity (RNA:protein ratio) and efficiency (protein synthesized:RNA ratio) of the translational process (Davis et al., 1991; Burrin et al., 1997). Chronic alterations in PS are the result of different numbers of ribosomes, whereas variations in translational initiation cause acute changes (Kimball and Jefferson, 1988).

**Jejunal Protein Synthesis.** Gastrointestinal tissues represent only 5% of BW, but because of their inherently high rates of metabolism, they represent a disproportionate fraction (ca. 15 to 35%) of whole-body oxygen consumption and protein turnover (McNurlan and Garlick, 1980). Compared with studies in neonatal lambs (Attaix et al., 1986; Patureau Mirand et al., 1990) and pigs (Burrin et al., 1992, 1996, 1997), PS rates in the intestines of calves were lower (16 to 26%). Possibly the time factor is of importance, although the calves were perfused for only 15 min, whereas the lambs in the study of Attaix et al. (1986) were perfused for 30 min. However, PS rates in the duodenum were higher in 3-wk-old pigs that were infused with $L$-[13C]Val using exactly the same injection procedure as we followed in the present study (Sève et al., 1993). In studies of Patureau-Mirand et al. (1990) and Burrin et al. (1992), the rapid protein accretion was attributed to the combined effects of a stimulation of PS by colostral growth factors and the absorption (by endocytosis) and retention in the enterocytes of ingested colostral proteins, such as immunoglobulins in enterocytes and, as shown by Burrin et al. (1992), a reduction in the rate of protein degradation in intestinal tissue. However, PS rates in the jejunum were independent of feeding colostrum or formula without or with added vitamin A, Lf, or vitamin A plus Lf. The period without food (lasting for about 12 h) before euthanasia may be an explanation for the moderate PS rates in the present study because a reduced energy and protein intake rapidly decreases PS rates (Kimball et al., 2000). In addition, PS and protein degradation could be reduced by both the lack of luminal substrate availability and the potential downregulation of trophic stimuli resulting from an absence of luminal nutrients (Stoll et al., 2000). However, the PS was in the range of estimates of cell turnover of small intestinal villus enterocytes (25%/d; Reeds et al., 1993).

**Hepatic Protein Synthesis.** Kimball et al. (2000) showed that hepatic PS was depressed after a short-term fasting period, but was rapidly stimulated in response to feeding. In the present study, the calves were without food for about 14 h before they were euthanized. This may explain the relatively low hepatic PS rates in the present study compared with others (Sève et al., 1993). The AA derived from intestinal absorption can stimulate hepatic PS and are a primary stimulus for liver PS in neonates (Davis et al., 1998a; O’Connor et al., 2004). Deprivation of essential AA or AA imbalances...
cause disaggregation of polysomes and decrease hepatic PS rates (Pronczuk et al., 1970). In fasted animals, the decrease in PS in the liver is due to roughly equal decreases in ribosome content, polynosome activity, and the proportion of ribosomes in polyribosomes (Henshaw et al., 1971).

**Correlations Between Protein Synthesis and Other Traits.** There was a positive correlation between FSR_{Liv} with plasma urea concentrations. This was not unexpected because plasma urea levels are mainly the result of the balance between degradation and production of proteins under the condition that there is no kidney disease (which was unlikely the case because calves in the present study were healthy). The FSR_{jej} was positively correlated with crypt depth, which can be taken as an expression of the potential capacity of the intestinal epithelial cell proliferation rate (Blättler et al., 2001; Bittrich et al., 2004). In accordance, FSR_{jej} also correlated with cell proliferation in the villus epithelium (ratio of BrdU-labeled cells:total villus cells) and significantly with crypt cell proliferation (BrdU-labeled crypt cells:total crypt cells), indicating that cell renewal in the GIT is mainly dependent on the crypts. Furthermore, the thickness of the intestinal muscularis externa, which is rich in proteins, was also correlated with FSR_{jej}. The positive associations of all these functions can be considered to depend on PS. On the other hand, FSR_{jej} was negatively correlated with villus height and negatively with the villus height: crypt depth ratio. This is not surprising in view of the findings of Bittrich et al. (2004), who found that crypt depths and crypt cell proliferation increased, whereas villus sizes decreased in the jejunum during the first 8 d of life of neonatal calves. In addition, the data on PS rate are in agreement with the data of Blättler et al. (2001), who showed that villus sizes in neonatal calves were negatively associated with epithelial cell proliferation rates, expressing negative feedback control of intestinal epithelial growth and a negative association of jejunal PS with these histological traits.

The FSR_{Liv} was negatively correlated with plasma total protein and albumin concentrations. The finding of a negative association of FSR_{Liv} with plasma albumin concentrations was surprising and is unlikely to be the result of the period without food (lasting for about 12 h) before euthanasia. Thus, albumin synthesis in sheep accounts for up to 17% of total liver PS in the fed condition and may fall to 8% during moderate fasting (Connell et al., 1997); that is, a positive rather than a negative association of FSR_{Liv} with plasma albumin concentrations could be expected. Because total protein levels were also negatively associated with FSR_{Liv}, changes in the plasma water content may have inverted the positive association between FSR_{Liv} and plasma albumin concentrations.

**CONCLUSIONS**

The primary objective of the present study was to determine the fractional protein synthesis rates in the jejunum and liver of neonatal calves and to determine the potential influence of vitamin A and Lf within this process. There were moderate PS rates in the jejunum and liver of neonatal calves. Significant positive correlations were found between the FSR_{jej} with the villus height: crypt depth ratio and with intestinal cell proliferation. There were unexpected significant negative correlations between FSR_{Liv} and plasma albumin levels. The exhalation of 14C and the decrease of L-[13C]Val in plasma changed over time, but there were no significant effects of feeding colostrum or formula without or with added vitamin A, Lf, or vitamin A plus Lf on the PS and no interactions between vitamin A and Lf.

**ACKNOWLEDGMENTS**

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