

Digestion of colostrum by the preruminant calf: digestibility and origin of undigested protein fractions in ileal digesta

René TOULLEC^{a*}, Jean-Paul LALLÈS^a,
Jean-François GRONGNET^a, Didier LEVIEUX^b

^a Unité Mixte de Recherche sur le Veau et le Porc, INRA/ENSAR,
65 rue de Saint-Brieuc, 35042 Rennes Cedex, France

^b Station de Recherche sur la Viande, INRA, Theix, 63122 Saint-Genès-Champanelle, France

(Received 29 September 1999; accepted 12 September 2000)

Abstract — Four diets (SMP, ML, DC and DCLC) containing 24, 18, 53 and 19% crude protein on a dry matter basis, respectively, were given to ileo-cæcal cannulated preruminant calves. In the SMP diet, protein was provided by skim milk powder. The ML diet was a mixture of raw whole milk and lactose. The DC and DCLC diets were first milking colostrum diluted with water (DC) or water, lactose and UHT cream (DCLC). The apparent ileal digestibility of N was low with the DC and DCLC diets (0.71 and 0.70 vs. 0.92 with the SMP and ML). Comparisons of AA profiles suggested that could be due to: (1) an increased loss of endogenous protein (+ 480 and + 200% for DC and DCLC, respectively), (2) an incomplete digestion of immunoglobulins G (IgG) which constituted 49 and 42% of digesta protein for DC and DCLC. The proportion of immunoreactive IgG found by radial immunodiffusion was similar for DC digesta (47%) but lower for DCLC (6%). Indeed, immunoblotting of non-reduced protein showed intact and/or almost intact IgG were more abundant for DC than DCLC. However, in reduced samples, intact and/or slightly degraded γ heavy chains were abundant for both. In contrast, only traces of IgG were detected in SMP and ML digesta.

immunoglobulin / digestion / calf

Résumé — **Digestion du colostrum chez le veau préruminant : digestibilité et origine des fractions indigérées à la fin de l'iléon.** Quatre aliments (SMP, ML, DC et DCLC), contenant respectivement 24, 18, 53 et 19 % de matières azotées par rapport à la matière sèche, ont été distribués à des veaux préruminants munis d'une canule ré-entrante iléo-cæcale. Dans l'aliment SMP, les protéines étaient apportées par de la poudre de lait écrémé. L'aliment ML était du lait frais additionné de lactose. Les aliments DC et DCLC étaient du colostrum de première traite additionné d'eau (DC) ou

* Correspondence and reprints

Tel.: (33) 2 23 48 53 51; fax: (33) 2 23 48 53 70; e-mail: toullec@roazhon.inra.fr

d'eau, de lactose et de crème UHT (DCLC). La digestibilité apparente de l'azote a été peu élevée avec les aliments DC et DCLC (0,71 et 0,70 vs. 0,92 avec SMP et ML). Les comparaisons de profils d'acides aminés indiquent que cela pourrait être dû à : (1) une augmentation des pertes endogènes (+ 480 et + 200 % avec les aliments DC et DCLC), (2) une digestion incomplète des immunoglobulines G (IgG) qui constituaient 49 et 42 % des protéines des digesta DC et DCLC. L'immunodiffusion radiale et les immunoempreintes confirment l'abondance des IgG dans les digesta DC et DCLC, sous des formes toutefois plus dégradées pour DCLC. En revanche, la concentration d'IgG était très faible dans les digesta SMP et ML.

immunoglobuline / digestion / veau

1. INTRODUCTION

In newborn ungulates, the early ingestion of a large amount of colostrum rich in immunoglobulins (Ig) is essential to get passive immunity [18]. In a first milking cow's colostrum, Ig represent approximately 50% of total protein [1], IgG 88% of total Ig and IgG₁ 95% of total IgG [29]. IgG are compact globular proteins with a Mr ~ 150 to 163 kg·mol⁻¹, made up of two γ heavy chains (Mr ~ 52 to 59 kg·mol⁻¹) and two λ or κ light chains (Mr ~ 23 to 27 kg·mol⁻¹) [5]. The heavy chains are disulfide-bonded together and to each light chain. Colostrum is also a source of nutrients that provides for the immediate requirements of the animals. It is particularly true for piglets, that have less energy stores at birth than ruminants, although their expenditures are higher [17]. Besides, colostrum contains numerous active substances, such as growth factors and hormones, which can have biological effects in newborns [12]. During the first 24 to 48 h following birth, Ig are largely absorbed in intact form, owing to the permeability of the intestinal epithelium to macromolecules [18]. The presence of antiproteases in colostrum has been considered to be a favoring factor [25]. However, their activity would be only 5% of that of raw soyabean [10]. The other proteins of colostrum can also be absorbed, but their circulating concentrations are much lower than that of Ig:

for example, 8 h after the first meal of colostrum given to calves 2 h after birth, the β -lactoglobulin to IgG ratio was 100 times lower in the blood plasma than in the colostrum (I. Nunes do Prado and R. Toullec, unpublished results). That was probably due to the lesser sensitivity of IgG to hydrolysis in the digestive tract [35] and to the rapid urinary clearance of small proteins [14]. The resistance of IgG to hydrolysis is probably linked to their compact globular structure. In newborn calves given colostrum, intact IgG₁ and their F(ab')₂ fragments have been detected in faeces [15]; however, the real digestibility of total IgG assayed by radial immunodiffusion was high (0.93) during the first 3 days from birth [10]. In contrast, little data is available on the subsequent efficiency of their digestion, when the permeability of the intestinal epithelium to macromolecules is very low. Bovine colostrum surplus are sometimes utilized as a milk replacer in weaning calves. The apparent digestibility of nitrogen (N) for a mixture of colostrum collected from the first two to six milkings was low during the third week of life: 0.81 [34], versus 0.93 for skim milk powder [9]. In adult men, a considerable part (0.19) of ingested bovine Ig was still in immunoreactive form at the end of the ileum [26]. To analyze further the reasons of these low values, we studied, in at least 2 month-old preruminant calves, the ileal digestion of the first milking colostrum. The first milking colostrum was used because it is richer in Ig than those

collected during the following milkings [18]. The origin of the undigested protein fractions was estimated by methods based on the comparison of amino acid (AA) profiles and by immunochemical techniques.

2. MATERIALS AND METHODS

2.1. Diets

Four diets (SMP, ML, DC and DCLC) were utilized (Tab. I). The SMP diet was a classical milk replacer, in which protein was provided exclusively by skim milk powder; it was given to the calves after dilution in lukewarm water (170 g dry matter (DM)·kg⁻¹ milk substitute). The other three diets were made up in order to get the same DM content. The ML diet was fresh whole milk enriched in lactose; it contained 180 g crude protein (CP)·kg⁻¹ DM. The DC and DCLC diets were prepared from first milking colostrum fractionated into 1 litre flasks and kept at -18 °C until use. Whole milk and colostrum were collected from Holstein cows (UMR production de lait, INRA/ENSAR, Rennes). The amount required for each meal was transferred at + 4 °C about 12 h before, care having been taken to choose flasks coming from many cows, in order to limit the variation of the composi-

tion of the final mixture. The DC diet was obtained by diluting colostrum with lukewarm water; its CP content was very high (526 g·kg⁻¹ DM). The DCLC diet was prepared by mixing colostrum, lactose, UHT cream and lukewarm water; it was made up to get a CP content close to that of the ML diet.

2.2. Animals, feeding and digesta collection

Six female Holstein calves were fitted with an abomasal catheter and a re-entrant ileo-cæcal canula between 8 and 10 weeks of age [11]. One to 2 weeks post-surgery, calves started to receive the experimental diets by means of abomasal infusion twice daily. The amount of DM infused (56 g·kg⁻¹ live weight^{0.75}·day⁻¹) was adjusted weekly. Two of the calves were given first the SMP diet and then the DC diet. Each experimental period lasted 7 days, and total digesta were collected during the last 4 days and treated as previously described [3]. The other four calves should have received the ML, DC and DCLC diets in different orders. However, because the colostrum stock was limited, the DC diet could be given to only two of them, and the duration of digesta collection was reduced to 2 days.

Table I. Chemical composition of the diets (% of dry matter).

Diet	SMP ^a	ML ^b	DC ^c	DCLC ^d
Crude protein (N × 6.38)	23.8	17.9	52.6	18.8
Fat	21.4	22.4	29.5	25.6
Ash	6.6	4.1	4.8	2.2
Nitrogen free extract	48.2	55.6	13.1	53.4

^a 65.4% spray-dried skim milk powder + 19.8% tallow + 8.0% pregelatinized starch + 3.7% lactose + 3.1% mineral and vitamin premix (for details, see [3]). ^b 95.8% whole milk + 4.2% lactose. ^c 69.8% first milking colostrum + 30.2% water. ^d 25.8% first milking colostrum + 7.63% lactose + 7.01% UHT cream (containing 35% fat) + 59.56% water.

2.3. Chemical analysis

Dry matter, nitrogen, fat, ash and AA were analyzed in the diets and digesta as previously described [3]. IgG were assayed by radial immunodiffusion, electrophoresis and immunoblotting, using total bovine serum IgG (I-5506 from SIGMA) as the standard. Radial immunodiffusion was conducted as described elsewhere [19], on fractions extracted in water for 1 h at 37 °C, with a hyperimmune serum prepared against the standard IgG. For the electrophoresis, proteins were extracted in borate buffer (0.1 mol·L⁻¹ HBO₃, 0.15 mol·L⁻¹ NaCl, pH 8.0) for 1.5 h at room temperature [4]. Concentration of protein in the solutions was determined according to Lowry et al. [20], using bovine serum albumin as the standard. Electrophoresis (SDS-PAGE) was carried out on mini-gels (80 × 90 mm) [16], with Tris-glycine buffer (25 mmol·L⁻¹ Tris, 192 mmol·L⁻¹ glycine, pH 8.3), a 12.5% acrylamide separating gel and a 4% acrylamide stacking gel. When required, dithiothreitol (200 mmol·L⁻¹) was used as the reducing agent. Molecular weight standards were from Pharmacia Biotech (reference 17-0446-01). Loadings were 10 µg of protein per track for pure products, 40 µg for colostrum and 200 µg for the other diets and digesta. Electrophoresis was performed for 1.5 h at 40 mA. The gels were stained for protein using Coomassie blue R250. After electrophoresis, the gels were equilibrated for 15 min in the transfer buffer and proteins were electro-transferred (1 h, 100 mA) to nitrocellulose membranes in Tris-glycine buffer containing 0.1% SDS (w/v) and 20% methanol (v/v). Transfer was monitored by staining membranes with Ponceau red. Membranes were saturated using 5% (w/v) skim milk powder in Tris buffer (20 mmol·L⁻¹ Tris, 137 mmol·L⁻¹ NaCl, pH 7.6) for 2 h at room temperature. They were incubated overnight with optimal dilutions of specific antibodies or hyperimmune sera, including affinity-purified rabbit polyclonal anti total bovine IgG

(B-5645 from SIGMA), sheep anti IgG₁-Fc and rabbit anti IgG-Fab sera (D. Leveux, INRA Theix), and two mouse monoclonal IgG₁ anti λ or κ bovine light chain (Big 43 A and Big 501 E from REALEF). After washing, they were incubated for 2 h with appropriate horse-radish peroxidase conjugated antibodies diluted at 1/1000 (v/v) in Tris buffer. Finally, immunolabelling was revealed by incubating membranes in Tris buffer (containing 1.85 mmol·L⁻¹ 3,3' diaminobenzidine tetrahydrochloride, 5.35 mmol·L⁻¹ hydrogen peroxide, 0.223 mmol·L⁻¹ NiCl₂ 6H₂O), for 5 to 7 min.

2.4. Statistical analysis

The SMP and ML diets gave similar values; therefore, they were put together to be the control diet. The realized experimental design did not allow a global statistical analysis of the digestibility data, but only paired t test between the control diet on the one hand and the DC or DCLC on the other hand.

Two methods based on the comparison of the AA profile of digesta with that of dietary, endogenous and microbial proteins were used to evaluate the origin of the digesta proteins. The first method only allows single comparisons between pairs of proteins by calculating the distance of χ^2 [11] as follows:

$$\chi^2 = 16 \frac{\sum[(AA_{ik} - AA_{jk})^2 / (AA_{ik} + AA_{jk})]}{2},$$

where AA_{ik} and AA_{jk} were the respective percentages of AA_k in the sum of the assayed AA (glutamic acid excluded, see Sect. 3.2.), in the proteins i and j; k, which represented the different AA, varied between 1 and 16. The larger the value of χ^2 , the greater the difference between the compared proteins. The second method [7] allows us to estimate the proportions of dietary, endogenous and bacterial proteins

which could be the main constituents of digesta protein. It uses a multiple regression analysis to establish the theoretical mixture of the reference proteins taken into account which minimizes the χ^2 distance with regard to the AA composition of digesta. It provides a statistical significance for the fit between the theoretical mixture and the digesta protein, as well as for the contribution of the reference proteins to the theoretical mixture. The composition of the diets, as well as literature data on milk Ig [8] were used for dietary protein. The mean composition of axenic lamb faeces [6] and calf meconium [9] was used as a model of undigested endogenous protein. The mean composition of sheep [21] and pig [22] faecal bacteria was chosen to represent the composition of gut bacteria.

2.5. Other calculations

Endogenous N or AA losses can be distributed between two fractions: a non-specific fraction (NSEL) resulting from the normal activity of the digestive tract, and a specific fraction (SEL) depending on the characteristics of the diet [27]. In calves given diets based on milk protein, N and AA escaping digestion in the small intestine appear to be exclusively from endogenous origin [11, 31]; since the undigested amounts and their AA composition do not change with the protein content of the diet [24], they were considered to represent NSEL. The respective amounts of total endogenous ($TEAA_{DC \text{ or } DCLC}$) and dietary ($DAA_{DC \text{ or } DCLC}$) AA in DC and DCLC digesta were estimated from the theoretical mixtures of control digesta and milk Ig that presented the best fit with DC and DCLC digesta (see Sect. 4.2.). The $SEL_{DC \text{ or } DCLC}$ of AA, the true digestibility ($TD_{DC \text{ or } DCLC}$) of N and AA, and the real digestibility ($RD_{DC \text{ or } DCLC}$) of AA with the DC or DCLC diet were calculated according to the following equations, where the items on the

right side were in $g \cdot kg^{-1}$ DM intake:

$$SEL_{DC \text{ or } DCLC} = TEAA_{DC \text{ or } DCLC} - NSEL \quad (1)$$

$$TD_{DC \text{ or } DCLC} = [I_{DC \text{ or } DCLC} - (TU_{DC \text{ or } DCLC} - NSEL)] / I_{DC \text{ or } DCLC} \quad (2)$$

where $I_{DC \text{ or } DCLC}$ was the amount of intake and $TU_{DC \text{ or } DCLC}$ the total undigested amount with the DC or DCLC diet

$$RD_{DC \text{ or } DCLC} = (I_{DC \text{ or } DCLC} - DAA_{DC \text{ or } DCLC}) / I_{DC \text{ or } DCLC} \cdot (3)$$

3. RESULTS

All the calves were healthy during the experiment. However, one of them had an abnormally high flow of digesta with the ML diet and the corresponding values were discarded.

3.1. Nutrient digestibility

Apparent ileal digestibility values varied generally much more with the DC and DCLC diets than with the control (Tab. II). Therefore, when the differences were great, conditions of variance homogeneity were never met to assess the significance of the differences between the control diet and the DC or DCLC. However, as far as N and AA were concerned, the physiological significance of the differences was supported by the results obtained about the origin of the undigested fractions (cf. Sects. 3.2. and 3.3.). Apparent ileal digestibility values of DM, organic matter, total N, AAN (Tab. II) and individual AA (data not shown) were much lower with the DC and DCLC diets than with the control. For example, values for total N were 0.71, 0.70 and 0.92, respectively. Values for fat were similar with the DC and control diets, but were much lower with the DCLC. In contrast, values for nitrogen free extract (NFE) were lower with the DC diet than with the other two diets.

Table II. Ileal digestibility of feed constituents (means ± SE).

	Diet		
	Control ^a	DC	DCLC
Number of measurements	5	4	4
Apparent digestibility:			
– dry matter	0.93 ± 0.010	0.79 ± 0.029	0.85 ± 0.028
– organic matter	0.94 ± 0.010	0.80 ± 0.026	0.86 ± 0.026
– total nitrogen	0.92 ± 0.007	0.71 ± 0.051	0.70 ± 0.076
– AAN ^b	0.94 ± 0.006	0.69 ± 0.072	0.74 ± 0.071
– fat	0.94 ± 0.024	0.94 ± 0.010	0.77 ± 0.066
– nitrogen free extract	0.95 ± 0.008	0.81 ± 0.036	0.95 ± 0.069
True digestibility ^c :			
– total nitrogen		0.74 ± 0.054	0.78 ± 0.081
– AAN ^b		0.72 ± 0.075	0.79 ± 0.071
Real digestibility ^c :			
– AAN ^b		0.83 ± 0.013	0.90 ± 0.026

^a SMP + ML. ^b Nitrogen of assayed amino acids, except amide-N of asparagine and glutamine. ^c For the calculations, see Section 2.5.

3.2. AA composition of diets and ileal digesta

Table III shows the AA composition of diets and digesta. As expected, the DC and DCLC diets had very similar AA compositions (data not shown), resulting in a small distance of χ^2 (2). Compared to the control, they contained more threonine, serine, glycine and cystine, and less isoleucine and leucine ($\chi^2 = 49$). Digesta from one of the calves had a much larger glutamic acid content than the others (31.0 g·100 g⁻¹ assayed AA versus 14.7 to 16.9 with the control diet). That reflected a peculiarity for the absorption and/or the metabolism of that AA in the gut wall of that animal. Therefore, the diet effect was evaluated by comparing AA compositions of diets and digesta expressed as percentages of the sum of the assayed AA, glutamic acid excluded. Digesta

protein always contained more threonine, serine, glycine, alanine and cystine, but less of the other AA, except valine, lysine and arginine than the corresponding diets; that resulted in great distances of χ^2 (≥ 69). In contrast, differences between digesta were smaller ($\chi^2 \leq 35$), although DC and DCLC digesta contained more serine and less alanine than control digesta.

All digesta were very different from gut bacteria ($\chi^2 \geq 155$) (Tab. IV). DC and DCLC digesta differed more from the undigested endogenous protein than did control digesta ($\chi^2 \geq 72$ versus 48). Also, they presented a less satisfactory fit with theoretical mixtures of endogenous and bacterial proteins ($\chi^2 \geq 50$ versus 19). In contrast, they were closer to milk Ig ($\chi^2 \leq 45$ versus 88), suggesting they contained a high proportion of dietary protein. The protein of DC and DCLC digesta could be a mixture of the

Table III. Amino acid (AA) composition of diets and digesta (g·100 g⁻¹ assayed AA).

Item	Diet (means)		Digesta (means ± SE)		
	Control ^a	DC + DCLC	Control ^a	DC	DCLC
Asp	8.09	8.49	9.39 ± 0.479	9.13 ± 0.489	9.37 ± 0.381
Thr	4.32	6.06	8.47 ± 0.448	8.07 ± 0.125	8.18 ± 0.743
Ser	5.52	7.27	7.11 ± 0.496	8.25 ± 0.508	8.76 ± 0.670
Glu	20.77	17.02	18.57 ± 3.307	19.31 ± 1.595	14.98 ± 0.815
Pro	9.44	8.81	6.98 ± 0.193	7.87 ± 0.332	7.53 ± 0.079
Gly	1.99	2.92	4.79 ± 0.156	4.60 ± 0.317	4.37 ± 0.347
Ala	3.46	3.82	5.49 ± 0.366	4.02 ± 0.121	4.60 ± 0.098
Val	6.23	6.63	6.10 ± 0.292	6.39 ± 0.133	7.21 ± 0.075
Cys	0.81	1.77	3.15 ± 0.233	2.96 ± 0.209	3.07 ± 0.125
Met	2.21 ^b	2.12	1.28 ± 0.081	1.02 ± 0.077	1.49 ± 0.239
Ile	5.03	4.19	4.00 ± 0.201	3.10 ± 0.186	3.90 ± 0.336
Leu	9.29	8.31	6.42 ± 0.402	6.26 ± 0.564	7.54 ± 0.337
Tyr	4.95	5.02	3.82 ± 0.314	3.94 ± 0.427	4.35 ± 0.313
Phe	4.84	4.47	3.78 ± 0.232	3.21 ± 0.244	4.00 ± 0.210
Lys	7.25 ^b	6.95	4.80 ± 0.375	6.59 ± 0.367	5.13 ± 0.570
His	2.45	2.28	2.10 ± 0.123	2.06 ± 0.110	1.83 ± 0.083
Arg	3.41	3.91	3.79 ± 0.276	3.25 ± 0.205	3.70 ± 0.089
Σ AA ^c	109.8	106.0	81.8 ± 4.28	107.2 ± 3.10	91.4 ± 1.67
AAN ^d	86.7	85.5	66.1 ± 2.87	86.6 ± 2.64	73.8 ± 1.41

^a SMP + ML. ^b Supplements excluded for SMP. ^c Sum of assayed AA (g·16 g⁻¹ N). ^d N of assayed AA, except amide-N of asparagine and glutamine (% of total N).

endogenous and bacterial proteins found in control digesta plus the dietary protein. Indeed, theoretical mixtures of control digesta (0.77 or 0.73) with the DC or DCLC diet (0.23 or 0.27) resembled slightly more DC or DCLC digesta than did control digesta alone ($\chi^2 = 28$ or 12 versus 35 or 19). However, better fits were obtained with mixtures including milk Ig, instead of the total dietary protein ($\chi^2 = 16$ or 8 with 0.46 or 0.37 Ig plus 0.54 or 0.63 control digesta).

3.3. IgG immunoreactive material in diets and ileal digesta

Immunoreactive IgG concentrations assayed by radial immunodiffusion were 11, 468 and 481 g·kg⁻¹ protein (protein = sum of the assayed AA × 0.9293, for taking into account the peptidic bonds) in the control, DC and DCLC diets, respectively. They were not measurable in any control and in two DCLC digesta. They were moderate (70 and 127 g·kg⁻¹ protein) in the other two

Table IV. Distances of χ^2 between ileal digesta protein and various proteins¹.

Digesta	Control ²	DC	DCLC
Control digesta (Cdg)	-	35	19
DC digesta		35	-25
DCLC digesta	19	25	-
Control diet	266	248	217
DC diet		117	94
DCLC diet		106	90
Immunoglobulins (Ig) ³	88	41	45
Undigested endogenous protein (UEP) ⁴	48	72	78
Gut bacteria ⁵	155	238	188
0.67 UEP ^b + 0.33 gut bacteria ^b	19 ^a	-	-
0.77 Cdg ^b + 0.23 DC diet	-	28 ^a	-
0.73 Cdg ^b + 0.27 DCLC diet ^b	-	-	12 ^a
0.54 Cdg ^b + 0.46 IgG ^b	-	16 ^a	-
0.63 Cdg ^b + 0.37 IgG ^b	-	-	8 ^a

¹ For the calculation of the distance of χ^2 , see Section 2.4. ² SMP + ML. ³ Milk immunoglobulins [8]. ⁴ Mean composition of axenic lamb faeces [6] and calf meconium [9]. ⁵ Mean composition of pig [21] and sheep faecal bacteria [20]. ^{ab} $P < 0.001$ for either the fit between ileal digesta and the theoretical mixture^a or the contribution of the constituent to the mixture^b.

DCLC digesta, but were very high in all DC digesta ($470 \pm 134 \text{ g}\cdot\text{kg}^{-1}$ protein, mean \pm SE).

The results of immunoblotting assays are summarized in Table V. Mr values preceded by “>” were values that could not be calculated precisely because they were much larger than the largest standard ($94 \text{ kg}\cdot\text{mol}^{-1}$) used in SDS-PAGE. The polyclonal antibodies and the two hyperimmune sera directed against IgG, IgG₁-Fc or IgG-Fab, respectively, recognized only a polypeptide of Mr $\sim 55 \text{ kg}\cdot\text{mol}^{-1}$ in the reduced sample of the total bovine serum IgG used as standard; that suggested they were specific of the γ heavy chains whose Mr is ~ 52 to $59 \text{ kg}\cdot\text{mol}^{-1}$ in bovine IgG [5]. Only a major polypeptide was also recognized by them in the DC and DCLC diets, but its Mr

was greater ($\sim 62 \text{ kg}\cdot\text{mol}^{-1}$) than for the standard IgG. The reason for this discrepancy is not known. In contrast, three main polypeptides (~ 32 to $58 \text{ kg}\cdot\text{mol}^{-1}$) were found in the corresponding digesta. Besides, three faint bands (~ 27 to $42 \text{ kg}\cdot\text{mol}^{-1}$) were detected in the diets and two ($\leq 27 \text{ kg}\cdot\text{mol}^{-1}$) in digesta. In non-reduced samples, three major bands (Mr > 138 or ~ 61 and $57 \text{ kg}\cdot\text{mol}^{-1}$, respectively) and two faint bands (~ 110 and $95 \text{ kg}\cdot\text{mol}^{-1}$) were present in the DC and DCLC diets. Five major bands (~ 32 to $> 133 \text{ kg}\cdot\text{mol}^{-1}$) were present in DC digesta, but only two (~ 53 and $85 \text{ kg}\cdot\text{mol}^{-1}$) in DCLC. Two to six faint bands (~ 21 to $> 126 \text{ kg}\cdot\text{mol}^{-1}$) were detected in DC and DCLC digesta. Only faint bands were apparent in reduced or non-reduced samples for control digesta. Finally,

Table V. Mr (kg·mol⁻¹) of the immunoreactive fractions detected by immunoblotting with polyclonal anti IgG antibodies and two hyperimmune anti IgG₁-Fc or anti IgG-Fab sera in diets and digesta¹.

Conditions of electrophoresis	reducing	non-reducing
Bovine serum IgG	55^a	> 146 (130, 119, 93, 59) ^b
DC and DCLC diets	62 (42, 38, 27)	> 138, 61, 57 (111, 95)
Control ^b digesta	(58, 31)	(> 126)
DC digesta	57, 41, 33 (27, 16)	> 133-138, 83, 63, 48 (> 115, 88, 52, 32, 25)
DCLC digesta	61, 43, 33 (27, 16)	55, 50 (> 120, 110, 93, 34)

¹ With the monoclonal anti- λ or κ light chains: a marked band (Mr ~ 26 kg·mol⁻¹) in the DC diet and digesta, a faint band (Mr ~ 19 kg·mol⁻¹) in DC digesta (DCLC, SMP and ML digesta not assayed for light chains).

^a^bBands of **strong**^a or **faint**^b intensity.

in reduced samples of the DC diet and digesta, both monoclonal anti λ or κ light chain showed a unique major polypeptide with a Mr ~ 26 kg·mol⁻¹ similar to that of intact chains [5]; a faint band (~ 19 kg·mol⁻¹) was also detected in digesta. Light chains were not assayed in DCLC and control digesta.

4. DISCUSSION

4.1. Nutrient digestibility

In the present experiment, comparisons of apparent digestibility values are partially hampered by differences between the diets, especially for their NFE, fat, and CP contents. The amounts of undigested NFE were similar with the three diets (25 to 28 g·kg⁻¹ DM intake), although the dietary concentration was much lower with the DC than with the others (Tab. I). Therefore, undigested NFE corresponded probably to endogenous products [2], whose total losses did not appear to change appreciably with

colostrum ingestion. The true and real digestibility of NFE, calculated as shown for N and AA in Section 2.5., appeared to be complete for the three diets. In contrast, the amount of undigested fat was 2.7 times higher with the DCLC diet than with the DC, although fat proportion was higher in the latter (Tab. I). Thus, fat provided by the UHT cream appeared to be less efficiently digested than fat from colostrum.

The amounts of N undigested per kg DM intake were 2.7 ± 0.3 , 24.1 ± 3.9 and 8.6 ± 2.2 g with the control, DC and DCLC diets, respectively. These variations were also larger than those of the amounts of intake (Tab. I), and the true digestibility of N and AAN was far from complete for the DCLC and especially the DC diets (0.79 and 0.72, respectively, for AAN) (Tab. II). Similar trends were observed for all the AA, especially cystine, threonine and glycine (data not shown). These incomplete true digestibility values were expected since the immunochemical assays demonstrated the presence of large amounts of colostrum IgG in digesta. Whether increased

losses of endogenous N could also be involved in the low true digestibility values recorded with the DC and DCLC diets was estimated from AA profile comparisons.

4.2. Origin of undigested protein

Immunoblotting showed IgG from the DC and DCLC diets were mainly intact molecules including two heavy and two light chains. Some free heavy and light chains were also present. A small part of IgG chains had also been partially hydrolyzed as shown by the faint bands at 95 and 110 kg·mol⁻¹, and between 27 and 42 kg·mol⁻¹, in non-reduced and reduced samples, respectively. That could be expected since the presence of low molecular weight forms of IgG has been described many times in secretions [5]. Intact and slightly digested heavy chains appeared to be abundant in DC and DCLC digesta. Intact and slightly digested light chains were also abundant, at least in DC digesta. As far as the heavy chains were concerned, the major fractions with Mr ~ 41 and 32 kg·mol⁻¹ could have resulted from the loss of one and two half-domains, respectively [30]. The light chain fraction with Mr ~ 19 kg·mol⁻¹ found in DC digesta could correspond to the loss of a linear zone not involved in a globular domain. The part of intact and/or almost intact IgG (Mr > 130 kg·mol⁻¹) appeared to be greater in DC digesta than in DCLC digesta. That was in agreement with the lower immunoreactivity of DCLC digesta compared to DC in radial immunodiffusion. Thus, IgG from the DC diet appeared to largely escape digestion in the small intestine without extensive degradation as was found by Kumano et al. [15] in newborn calves given colostrum. In contrast, undigested IgG appeared to be more extensively degraded with the DCLC diet.

From the theoretical mixtures of control digesta and Ig which presented the best fit with the DC and DCLC digesta (cf.

Sect. 2.4.), the total endogenous losses of AA were estimated to be approximately 76 ± 11 and 31 ± 11 g AA·kg⁻¹ DM intake for DC and DCLC, respectively. They were 4.8 ± 1.7 and 2.0 ± 1.3 times higher than the values observed in the same calves when given the control diet. Therefore, the DC and DCLC diets appeared to induce high specific losses of endogenous protein, as was also shown by a 2.3 and 1.3 times increased flow of mucins at the end of the ileum [23]. According to the metabolic cost estimated for the synthesis of endogenous protein [13], these specific endogenous losses could increase the maintenance requirement by 15 and 5%, respectively. Many dietary constituents (antinutritional factors, fibres, non-self proteins, etc.) have been shown to give rise to specific endogenous losses [28]. The present experiment suggests "self" proteins resistant to digestion could also have that effect. The involved mechanisms (mechanical dragging, hydrophobic interaction, etc.) remain to be established.

On these bases, the average amounts of undigested dietary AA would be 86 ± 27 and 16 ± 2 g·kg⁻¹ DM intake, i.e. 17 ± 6 and 10 ± 1% of total AA intake, with the DC and DCLC diets, respectively. Therefore, the real digestibility of total colostrum AAN appeared to decrease from 0.90 to 0.83 when the amount of intake increased twofold (Tab. II). A similar trend was observed for all the assayed AA, except methionine and isoleucine (data not shown). These differences should be considered cautiously, because they did not result from direct measurements. Nevertheless, they were supported by the presence of larger amounts of less degraded IgG in DC digesta as compared with DCLC digesta. In contrast, no decrease of the real digestibility was observed by doubling the proportion of soluble wheat protein and whey protein at the expense of skim milk protein in diets whose crude protein content was maintained at 219 g·kg⁻¹ DM [33]. That

suggested the protein content of the DC diet could be beyond the level up to which its real digestibility stayed constant. This phenomenon is more documented for starch whose digestibility decreases when its content increases in the diet [32].

4.3. Conclusion

This study demonstrates IgG from colostrum largely resist digestion up to the end of the ileum, even a long time after birth. The colostrum-based diets appeared also to induce a specific loss of endogenous protein. The extent of both phenomena depended on the amount of IgG intake, but remained significant when the protein concentration of the diet was reduced to 36% of its initial value in colostrum. Therefore, colostrum is a less efficient source of protein than mature milk. However, its IgG should partially keep their local protective effects along the digestive tract; that could be useful after the neonatal period to limit the development of enteric pathogens.

ACKNOWLEDGMENTS

Thanks are due to J. Quillet for collecting the bibliographical information, to S. Boussion, A. Cahu, F. Faucheux, H. Flageul, M. Formal, J. Lareynie, M. Lesné and G. Savary for technical assistance, and to the région de Bretagne, le département d'Ile-et-Vilaine and the district of Rennes for financial support.

REFERENCES

- [1] Alais C., Sciences du lait : principes et techniques laitières, SEPAIC, Paris, 1984.
- [2] Besle J.M., Pougheon M., Bayle E., Thivend P., Étude des glucides totaux et des glycoprotéines endogènes des contenus intestinaux chez le veau préruminant, *Reprod. Nutr. Dev.* 23 (1983) 25–39.
- [3] Branco-Pardal P., Lallès J.P., Formal M., Guilloteau P., Toullec R., Digestion of wheat gluten and potato protein by the preruminant calf: Digestibility, amino acid composition and immunoreactive proteins in ileal digesta, *Reprod. Nutr. Dev.* 35 (1995) 639–654.
- [4] Bush R.S., Toullec R., Caugant I., Guilloteau P., Effects of raw pea flour on nutrient digestibility and immune responses in the preruminant calf, *J. Dairy Sci.* 75 (1992) 3539–3552.
- [5] Butler J.E., Biochemistry and biology of ruminant immunoglobulins, *Prog. Vet. Microbiol. Immun.* 2 (1986) 1–53.
- [6] Combe E., Influence de la microflore sur la composition en acides aminés des fèces des agneaux, *C.R. Soc. Biol.* 170 (1976) 787–793.
- [7] Duvaux C., Guilloteau P., Toullec R., Sissons J.W., A new method of estimating the proportions of different proteins in a mixture using amino acid profiles: application to undigested proteins in the preruminant calf, *Ann. Zootech.* 39 (1990) 9–18.
- [8] Gordon W.G., Kalan E.B., Proteins of milk, in: Webb B.H., Johnson A.H., Alford J.A. (Eds.), *Fundamentals of dairy chemistry*, Avi Publishing Co., Westport, Connecticut, 1974, pp. 87–124.
- [9] Grongnet J.F., Patureau-Mirand P., Toullec R., Prugnaud J., Utilisation des protéines du lactosérum par le jeune veau préruminant. Influence de l'âge et de la dénaturation des protéines, *Ann. Zootech.* 30 (1981) 443–464.
- [10] Grongnet J.F., Grongnet-Pinchon E., Levieux D., Piot M., Lareynie J., Newborn calf intestinal absorption of immunoglobulins extracted from colostrum, *Reprod. Nutr. Dev.* 26 (1986) 731–743.
- [11] Guilloteau P., Toullec R., Grongnet J.F., Patureau-Mirand P., Prugnaud J., Sauviant D., Digestion of milk, fish and soya-bean protein in the preruminant calf: flow of digesta, apparent digestibility at the end of ileum and amino acid composition of ileal digesta, *Br. J. Nutr.* 55 (1986) 571–592.
- [12] Hammon H., Bühler C., Rossi G., Blum J.W., Effects of different colostrum supply on xylose absorption, small intestine morphology, and IGF and insulin receptors in neonatal calves, in: Blum J.W., Elsasser T., Guilloteau P. (Eds.), *Proc. of the symposium on growth in ruminants*, University of Berne, Berne, Switzerland, 1998, p. 290.
- [13] Huisman J., Prospects in gastrointestinal physiology in pigs and veal calves, in: Jansman A.J.M., Huisman J. (Eds.), *Nutrition and gastrointestinal physiology – today and tomorrow*, TNO, Wageningen, The Netherlands, 1999, pp. 85–103.
- [14] Kilshaw P.J., Slade H., Passage of ingested protein into the blood during gastrointestinal hypersensitivity reactions: experiments in the preruminant calf, *Clin. Exp. Immunol.* 41 (1980) 575–582.

- [15] Kumano Y., Kanamaru Y., Niki R., Arima S., Occurrence of bovine IgG₁ fragment in feces of newborn calf, *Jpn J. Zootech. Sci.* 47 (1976) 551–556.
- [16] Laemmli U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* 227 (1970) 680–685.
- [17] Le Dividich J., Herpin P., Rosarion-Ludovino R.M., Utilization of colostral energy by the newborn pig, *J. Anim. Sci.* 72 (1994) 2082–2089.
- [18] Levieux D., Transmission de l'immunité passive colostrale: le point des connaissances, in: Jarrige R. (Ed.), *Physiologie et pathologie périnatales chez les animaux de ferme*, INRA, Paris, 1984, pp. 345–369.
- [19] Levieux D., Dosage des IgG du lait de vache par immunodiffusion radiale semi-automatisée, pour la détection du colostrum et des laits de mammite ou de fin de gestation. I- Mise au point du dosage, *Lait* 71 (1991) 327–338.
- [20] Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [21] Mason V.C., The quantitative importance of bacterial residues in the non-dietary faecal nitrogen of sheep. I-Methodology studies, *Z. Tierphysiol. Tierernähr. Futtermittelkd.* 41 (1979) 131–139.
- [22] Mason V.C., Just A., Bech-Andersen S., Bacterial activity in the hindgut of pigs. II- Its influence on the apparent digestibility of nitrogen and amino acids, *Z. Tierphysiol. Tierernähr. Futtermittelkd.* 36 (1976) 310–324.
- [23] Montagne L., Lallès J.P., Toullec R., Calf intestinal mucin: isolation, partial characterization and measurement in ileal digesta using an enzyme-linked immunosorbent assay, *J. Dairy Sci.* 83 (2000) 507–517.
- [24] Montagne L., Toullec R., Lallès J.P., Quantitative and qualitative changes of endogenous nitrogen components along the small intestine of the calf, *J. Sci. Food Agric.* 80 (2000) 2123–2134.
- [25] Quigley J.D., Martin K.R., Dowlen H.H., Lamar K.C., Addition of soybean trypsin inhibitor to bovine colostrum: Effects on serum immunoglobulin concentrations in Jersey calves, *J. Dairy Sci.* 78 (1995) 886–892.
- [26] Roos N., Mahé S., Benamouzig R., Sick H., Rautureau J., Tomé D., N-15-labeled immunoglobulins from bovine colostrum are partially resistant to digestion in human intestine, *J. Nutr.* 125 (1995) 1238–1244.
- [27] Sève B., Henry Y., Protein utilization in the non-ruminants, in: Nunes A.F., Portugal A.V., Costa J.P., Ribeiro J.R. (Eds.), *Protein metabolism and nutrition*, INIA, Santarém, Portugal, 1996, pp. 59–82.
- [28] Sève B., Leterme P., Amino acid fluxes in the pig, in: Laplace J.P., Février C., Barbeau A. (Eds.), *Digestive physiology in pigs*, EAAP Publ. N° 88, INRA, Paris, 1997, pp. 304–315.
- [29] Silim A., Rekiq M.R., Roy R.S., Salmon H., Pastoret P.P., Immunité chez le fœtus et le nouveau-né, in: Pastoret P.P., Govaerts A., Bazin H. (Eds.), *Immunologie animale*, Flammarion Médecine-Sciences, Paris, 1990, pp. 197–204.
- [30] Strosberg D., Les immunoglobulines, in: Bach J.F. (Ed.), *Immunologie*, Flammarion Médecine-Sciences, Paris, 1986, pp. 236–309.
- [31] Tolman G.H., Beelen G.M., Endogenous nitrogen and amino acid flow in the terminal ileum of veal calves and true digestibility of skim milk, soluble wheat and soya isolate proteins, in: *Veal perspectives to the year 2000*, Fédération de la Vitellerie Française, Paris, 1996, pp. 191–207.
- [32] Toullec R., Veal production, in: Jarrige R., Béranger C. (Eds.), *Beef cattle production*, Elsevier, Amsterdam, The Netherlands, 1992, pp. 335–356.
- [33] Toullec R., Formal M., Digestion of wheat protein in the preruminant calf: ileal digestibility and blood concentrations of nutrients, *Anim. Feed Sci. Technol.* 73 (1998) 115–130.
- [34] Troccon J.L., Patureau-Mirand P., Gouet P., Conservation du colostrum, utilisation par le veau d'élevage, *Bull. Techn. CRZV Theix INRA* 31 (1978) 43–52.
- [35] Yvon M., Levieux D., Valluy M.C., Péliissier J.P., Patureau-Mirand P., Colostrum protein digestion in newborn lambs, *J. Nutr.* 123 (1993) 586–596.