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Digestion of milk protein and methanol-grown bacteria protein in the preruminant calf. II. Amino acid composition of ileal digesta and faeces and blood levels of free amino acids

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Summary. The aim of this trial was to study the balance of the amino acid digestion of milk and of methanol-grown bacteria in the terminal small intestine and the hindgut of the preruminant calf. Two diets (control and bacteria) were used. The protein of the control diet was furnished exclusively by skim-milk powder ; 50.5 p. 100 of the protein of the bacteria diet was supplied by methanol-grown bacteria, and the rest by skim-milk powder and synthetic amino acids.

Except for methionine, the apparent digestibility of all the amino acids assayed was lower in the terminal small intestine than in the whole digestive tract but the differences were usually less than for total nitrogen (table 5). The largest difference concerned cystine whose apparent digestibility increased from 76 p. 100 in the terminal ileum to 87 p. 100 in the faeces for the control diet, the corresponding values for the bacteria diet being 64 and 79 p. 100. The diaminopimelic acid of dietary bacteria did not seem to be absorbed in the small intestine but large quantities disappeared in the large intestine (table 7). Amino acid digestibility was higher for the milk than for the dietary bacteria, except for threonine and glycine in the terminal ileum and glycine and alanine in the faeces (table 5).

The ileal digesta were richer than the faeces in threonine, serine, proline and cystine and were poorer in methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and arginine (tables 2 and 3). These differences might result from a larger proportion of endogenous protein and a lower amount of gut bacteria protein in the digesta, which also contained more dietary protein than the faeces, at least with the bacteria diet, as shown by the quantities of diaminopimelic acid found with that diet. The respective proportions of dietary, endogenous and gut microbial proteins estimated in the terminal ileum were 16, 71 and 13 p. 100, respectively, with the control diet.

The lower digestibility of nitrogen from methanol-grown bacteria seemed due mostly to bacterial wall protein (especially peptidoglycan) resistance to digestion in the small intestine (table 3). However, the differences as compared to milk protein were small ; they did not permit us to show the unavailability of any particular amino acid (except for diaminopimelic acid) at the digestive or the metabolic level.

Introduction.

Protein digestibility in the preruminant calf differs depending on the source and the technological treatment of the protein (Toullec *et al.*, 1975). Due to large changes caused by the flora in the hindgut (Patureau-Mirand *et al.*, 1977 ; Toullec, Coroller and Patureau-Mirand, 1977), it is difficult to use the amino acid composition of the faeces to estimate the digestibility of the amino acids and the origin of apparently undigested protein. It is probable that the nitrogen absorbed in the large intestine is no longer in the form of amino acids, and that it is not utilizable by the calf, as observed in the pig (Zebrowska, Buraczewska and Horaczynski, 1978). We thus thought it interesting to study the amino acid composition of digesta protein sampled in the terminal small intestine, as shown in work on ruminants (Coelho da Silva *et al.*, 1972 ; Ben-Ghedalia *et al.*, 1974) and in pigs (see review by Rérat, 1978).

To study the digestive utilization of milk and methanol-grown bacteria proteins, we determined the amino acid composition of faeces and digesta sampled in the terminal ileum of preruminant calves given diets containing those proteins. These observations were completed by the measurement of postprandial free amino acid levels in the blood. Results on the overall digestibility of these diets in the terminal ileum and in the whole digestive tract have been reported earlier (Guilloteau and Toullec, 1980).

Material and methods.

Feeds, animals and sampling. — Two milk replacers (control and bacteria) containing 25.3 and 25.5 p. 100, respectively, of protein in relation to dry matter, were prepared. In the control diet the protein was supplied exclusively by skim-milk powder ; 50.5 p. 100 of the protein in the bacteria diet was provided by methanol-grown bacteria and the rest by skim-milk powder and synthetic amino acids. The bacteria (*Pseudomonas methylotropha*) constituted 16.5 p. 100 of the corresponding diet.

Four Friesian male calves were used to measure digestibility in the whole digestive tract. Five others were fitted with an indwelling cannula with its proximal part inserted into the terminal ileum and its distal part in the caecum. Two calves of each group were first fed the control diet and the others the bacteria diet. Four measurement periods were used from about 7 weeks of age ; the diets were reversed at the end of the second period.

Other details concerning the feeds, animals, feeding and sampling of the faeces and the digesta have been described by Guilloteau and Toullec (1980).

Analysis. — The amino acid composition of the protein was determined on a sample of dietary bacteria and of control diet and, for each diet, on two mean samples of faeces and ileal digesta. These mean samples were prepared for periods 2 and 4 from the individual samples of 2 calves given the same diet. Blood free amino acid concentrations were measured in one mean sample per diet, obtained at the end of the second period by mixing the samples taken between 3 1/2 and 4 hours after the morning meal from the jugular vein of 2 non-fistulated calves fed the same diet. Using ion-exchange resin chromatography, the amino acids of the bacteria, control diets, faeces and digesta were assayed after acid hydrolysis (Prugnaud and Pion, 1976) and the free amino acids of the blood after extraction (Pawlak and Pion, 1968).

The diaminopimelic acid of the dietary bacteria and of the mean samples of ileal digesta and faeces, prepared for the assay of other amino acids, was assayed by ion-exchange resin chromatography after performic acid oxidation and acid hydrolysis (Prugnaud, 1980).

Statistical computation. — The protein amino acid compositions were compared by calculating the distance of χ^2 as follows :

$$\chi^2 \text{ between proteins i and j} = 17 \sum_{k=1}^{17} \frac{(AAik - AAjk)^2}{\frac{AAik + AAjk}{2}}$$

AAik and AAjk are the respective percentages of amino acid k in the sum of the amino acid levels assayed (table 1) in proteins i and j ; k, which represents the various amino acids (except diaminopimelic acid), varied between 1 and 17. The larger the χ^2 value, the more difference there was between the compared proteins.

TABLE 1

Amino acid composition of dietary bacteria and diets

	g/16 gN			p. 100 of the sum of corrected levels of essential amino acids (1)		
	Control diet	Bacteria	Bacteria diet	Control diet	Bacteria	Bacteria diet
Aspartic acid	8.05	9.40	8.65	—	—	—
Threonine	4.85	4.55	4.65	9.89	11.50	10.41
Serine	5.25	3.35	4.25	—	—	—
Glutamic acid	23.20	10.45	16.60	—	—	—
Proline	10.40	3.55	6.85	—	—	—
Glycine	2.05	5.20	3.65	—	—	—
Alanine	3.30	7.25	5.25	—	—	—
Valine	6.50	5.50	5.95	13.25	13.90	13.27
Cystine	1.05	0.60	0.80	8.26	8.05	8.35
Methionine	3.00	2.60	2.95			
Isoleucine	5.35	4.25	4.75	10.91	10.70	10.61
Leucine	9.65	7.05	8.25	16.36	16.15	15.91
Tyrosine	5.10	3.20	4.10	12.00	12.00	12.00
Phenylalanine	5.10	3.60	4.30			
Lysine	8.10	6.25	7.65	16.52	15.75	17.13
Histidine	2.70	1.85	2.25	5.51	4.65	5.02
Arginine	3.60	4.90	4.25	7.30	7.30	7.30
Diaminopimelic acid	—	0.42	0.20	—	—	—
Sum of corrected levels of essential amino acids (1)		—		49.04	39.65	44.78

(1) Leucine level limited to 1.5 times that of isoleucine ; aromatic amino acid and arginine levels limited to 12 and 7.3 p. 100, respectively, of the sum of corrected levels of essential amino acids (tryptophane not assayed) (Pion, de Belsunce and Fauconneau, 1963).

Results.

Amino acid composition of bacterial protein (table 1). — Bacterial protein has a lower content of essential and semi-essential amino acids than cow's milk protein (39.65 and 49.04 p. 100, respectively, for the sum of the corrected contents). However, their essential and semi-essential amino acid balance, characterized by a large amount of threonine, a marked deficit of sulfur amino acids and a slight histidine deficit, resembled that of cow's milk. On the contrary, the non-essential amino acid composition of the bacterial protein was very different from that of milk protein since it was richer in alanine and glycine and poorer in serine, glutamic acid and proline.

Amino acid composition of ileal digesta and faeces. — The amino acid composition of ileal digesta and faeces depended little upon the measurement period (table 2) ; the χ^2 values obtained by comparing the digesta compositions of periods 2 and 4 were 5 and 14 when the animals were given the control and the bacteria diets, respectively ; these values were 12 and 7 when the faeces were compared. Composition differences were too small to be significant ($\chi^2 > 26$ for $P < 0.05$), and it was highly probable (50 to 99.5 p. 100, depending on the case) that they were similar at the two

TABLE 2

Amino acid composition of proteins in ileal digesta and of faeces (g/16 g N)

Protein source	Ileal digesta				Faeces			
Diet	Control		Bacteria		Control		Bacteria	
Measurement period	2	4	2	4	2	4	2	4
Aspartic acid	7.50	7.40	8.30	8.00	9.75	9.15	9.15	8.75
Threonine	8.05	7.40	5.55	5.55	5.20	4.50	4.45	4.30
Serine	5.30	4.90	4.20	4.05	4.50	3.90	3.80	3.55
Glutamic acid	12.45	12,70	11.10	12.80	12.65	12.55	10.15	10.05
Proline	6.30	5.85	4.20	4.05	4.20	3.65	3.25	3.00
Glycine	3.85	3.70	4.40	4.35	4.80	4.60	5.05	5.05
Alanine	3.60	4.00	5.90	6.20	5.75	6.25	5.75	6.45
Valine	4.65	4.85	4.30	4.35	5.80	5.70	5.05	5.15
Cystine	2.95	2.85	2.00	2.15	3.20	2.45	1.90	1.70
Methionine	0.90	1.00	1.25	1.15	2.15	2.25	2.25	1.90
Isoleucine	2.75	2.95	2.95	2.90	4.05	4.15	4.05	4.25
Leucine	4.80	4.75	5.15	5.00	6.60	6.15	6.55	6.45
Tyrosine	2.55	2.55	2.60	2.60	4.15	3.85	3.65	3.45
Phenylalanine	2.45	2.50	2.45	3.40	4.10	4.05	4.05	NA
Lysine	4.25	4.50	4.35	4.50	5.90	5.85	5.35	5.70
Histidine	1.70	1.55	1.70	1.55	1.90	1.35	1.75	1.50
Arginine	2.90	2.85	3.10	2.85	4.45	3.85	3.70	3.45
Diaminopimelic acid	0.21	0.16	2.10	2.00	0.42	0.67	0.42	0.50
N of assayed amino acids (1) (p. 100 of total N)	63.0	62.3	61.4	62.2	74.4	69.6	66.8	63.6

NA : Not assayed.
(1) Excluding diaminopimelic acid.

TABLE 3

Amino acid composition of diets, dietary bacteria, faecal bacteria, ileal digesta and faeces
(p. 100 of assayed amino acids, excluding diaminopimelic acid)

Proteins	Diet		Bacteria		Ileal digesta		Faeces			Additional undigested protein due to dietary bacteria ([3])	
	Control	Bacteria	Dietary	Faecal ([1])	Control	Bacteria	Control	Bacteria	Germ-free lambs ([2])	Ileum	Faeces
Aspartic acid....	7.51	9.11	11.25	11.4	9.72	10.94	10.90	11.28	10.30	14.62	12.24
Threonine	4.52	4.90	5.45	5.3	10.08	7.45	5.58	5.51	14.41	0	5.30
Serine	4.90	4.47	4.01	4.7	6.66	5.54	4.84	4.63	7.61	0.90	4.07
Glutamic acid...	21.63	17.42	12.51	12.3	16.42	16.03	14.54	12.73	14.32	13.36	8.04
Proline.........	9.70	7.21	4.25	3.9	7.93	5.54	4.52	3.94	8.27	0	2.41
Glycine	1.91	3.81	6.22	5.4	4.92	5.88	5.42	6.37	4.25	8.95	8.79
Alanine	3.08	5.54	8.68	6.7	4.96	8.12	6.93	7.70	5.14	19.32	9.66
Valine	6.06	6.25	6.58	6.5	6.17	5.81	6.64	6.43	5.56	3.99	5.89
Cystine	0.98	0.86	0.72	1.7	3.78	2.78	3.25	2.27	3.07	0	0
Methionine	2.80	3.08	3.11	2,4	1.24	1.61	2.54	2.61	1.50	2.87	2.81
Isoleucine	4.99	4.99	5.09	5.6	3.72	3.92	4.73	5.23	3.28	4.39	6.52
Leucine	9.00	8.68	8.44	8.8	6.23	6.82	7.35	8.20	5.50	8.44	10.34
Tyrosine	4.76	4.31	3.83	4.8	3.32	3.49	4.61	4.47	2.69	3.81	4.10
Phenylalanine ..	4.76	4.52	4.30	6.0	3.23	3.92	4.70	5.07	2.79	6.22	6.14
Lysine	7.55	8.05	7.48	6.9	5.71	5.94	6.78	6.97	5.03	6.32	7.43
Histidine	2.52	2.36	2.21	2.2	2.12	2.18	1.86	2.05	2.20	2.23	2.49
Arginine	3.36	4.44	5.86	5.0	3.75	4.00	4.78	4.50	4.07	4.58	3.77

([1]) Bacteria isolated in pig faeces by Mason, Just and Bech-Andersen (1976).
([2]) Results obtained by Combe (1976) in germ-free lambs fed cow's milk.
([3]) Additional undigested protein in relation to control diet. Diaminopimelic acid level in the additional undigested proteins (expressed in p. 100 of the alanine level) : 61.7 in ileal digesta and 4.4 in faeces.

periods. Thus, the mean compositions corresponding to the two periods may be used (tables 3 and 4).

TABLE 4

Computed χ^2 (1) *values comparing the amino acid composition* (in p. 100 of the sum of the assayed amino acids, diaminopimelic acid excepted) *of two protein compounds*

Protein source	Comparison of control and bacteria diets
Diets	87
Ileal digesta	74
Faeces	20

Protein source	Comparison of different protein compounds in the calves given	
	Control diet	Bacteria diet
Ileal digesta-faeces	152	71
Ileal digesta-diet	310	147
Ileal digesta-dietary bacteria	—	130
Ileal digesta-faecal bacteria (2), (3)	258	109
Ileal digesta-germ-free lamb faeces (4)	48	160
Faeces-diets	306	129
Faeces-dietary bacteria	—	44
Faeces-faecal bacteria (2), (3)	39	14

(1) $\chi^2 = 17 \sum_{k=1}^{17} \frac{(AAik - AAjk)^2}{\frac{AAik + AAjk}{2}}$.

(2) Bacteria isolated in pig faeces (Mason, Just and Bech-Andersen, 1976).

(3) χ^2 between faecal and dietary bacteria : 47.

(4) Germ-free lamb faeces (Combe, 1976).

The amount of nitrogen in the form of amino acids was lower in the digesta than in the faeces (a mean of 62.2 *vs* 68.6) (table 2). The digesta were richer in threonine, serine, proline and cystine but poorer in methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and arginine than the faeces (tables 2 and 3). Digesta diaminopimelic acid content was 2.9-fold lower than that of the faeces with the control diet ; it was much higher (4.5 times) with the bacteria diet.

With that diet, the faeces contained less glutamic acid, proline, and cystine and more glycine, alanine, isoleucine and leucine than with the control diet (tables 2 and 3) ; however, the differences were slight ($\chi^2 = 20$). The digesta contained less threonine, serine, proline and cystine and more glycine, alanine and phenylalanine ; these differences were greater than in the faeces, and the χ^2 value (76) suggested a high probability ($P > 0.99$) that those two compositions were different.

Faecal diaminopimelic acid levels were similar with both diets ; on the contrary, those of the digesta were about 11 times higher with the bacteria diet.

TABLE 5

Apparent digestibility of total nitrogen and amino acids (p. 100)

Part of digestive tract studied	Small intestine			Large intestine			Whole digestive tract			R (1)		
Protein	Control diet	Bacteria diet	Bacteria only	Control diet	Bacteria diet	Bacteria only	Control diet	Bacteria diet	Bacteria only	Control diet	Bacteria diet	Bacteria only
Total nitrogen	91.3	88.8	86.4	43.6	36.0	31.2	95.1	92.8	90.7	0.960	0.957	0.954
Nitrogen of assayed amino acids (2)	93.5	91.2	88.5	35.2	32.5	28.3	95.8	94.1	92.0	0.976	0.969	0.962
Nitrogen of assayed essential amino acids	93.9	92.1	89.9	31.5	33.9	19.6	95.8	94.7	91.9	0.980	0.972	0.978
Aspartic acid	91.9	89.5	87.5	28.4	29.7	30.4	94.2	92.6	91.1	0.976	0.966	0.960
Threonine	86.1	86.7	87.3	64.6	49.5	32.6	95.1	93.3	91.4	0.906	0.929	0.955
Serine	91.5	89.2	85.6	53.5	43.0	33.6	96.1	93.8	90.4	0.953	0.950	0.946
Glutamic acid	95.3	91.9	84.8	43.5	45.9	47.5	97.3	95.6	92.0	0.979	0.961	0.922
Proline	94.9	93.3	88.7	63.6	51.5	36.1	98.2	96.7	92.8	0.967	0.964	0.956
Glycine	83.9	86.5	87.5	29.7	26.1	24.3	88.7	90.0	90.5	0.946	0.961	0.966
Alanine	90.0	87.2	86.0	10.9	35.5	43.2	91.0	91.7	92.0	0.988	0.950	0.934
Valine	93.7	91.9	89.8	32.6	24.5	19.7	95.6	93.9	91.8	0.979	0.979	0.978
Cystine	75.9	71.5	64.1	45.1	44.5	43.8	86.8	84.2	79.8	0.875	0.849	0.803
Methionine	97.2	95.4	93.2	— 31.0	— 10.7	— 7.0	96.4	94.9	92.7	1.009	1.005	1.001
Isoleucine	95.4	93.1	90.4	18.8	09.2	3.5	96.2	93.8	90.8	0.991	0.993	0.996
Leucine	95.7	93.1	89.8	24.7	18.0	14.3	96.8	94.4	91.2	0.989	0.987	0.984
Tyrosine	95.6	92.9	88.7	11.5	12.6	13.3	96.1	93.8	90.2	0.995	0.990	0.983
Phenylalanine	95.8	92.4	87.8	7.2	11.4	13.3	96.1	93.3	89.4	0.997	0.991	0.982
Lysine	95.3	93.5	91.1	24.3	20.1	9.8	96.4	94.8	91.9	0.988	0.986	0.990
Histidine	94.8	91.3	88.0	43.7	36.0	31.2	97.0	94.8	91.7	0.976	0.969	0.959
Arginine	93.0	92.1	91.5	18.6	23.1	25.7	94.3	94.0	93.7	0.986	0.981	0.977
Diaminopimelic acid	—	— 07.9	— 04.2	— 66.6	85.6	91.0	—	84.5	90.7	—	—	—

(1) $R = \frac{\text{Apparent digestibility in terminal ileum}}{\text{Apparent digestibility in terminal digestive tract}}$.

(2) Excluding diaminopimelic acid.

Apparent amino acid digestibility (table 5). — Apparent nitrogen digestibility of the assayed amino acids was higher than that of total nitrogen in the whole digestive tract, and especially in the terminal small intestine where the differences were 2.2 and 2.4 p. 100, respectively, for the control and the bacteria diets. Apparent nitrogen digestibility of essential and semi-essential amino acids measured with the two diets, in the terminal ileum or large intestine, was similar to that of the total amino acids.

Whatever the diet, there was a high apparent digestibility of the various amino acids measured in the faeces or in ileal digesta, especially of glutamic acid, proline, methionine and lysine ; cystine, glycine and alanine digestibilities were lower. Amino acid digestibility in the terminal ileum was generally higher with the control than with the bacteria diet ; this was particularly true in relation to cystine (75.9 *vs* 71.5) and less so for glutamic acid, phenylalanine, histidine and alanine. However, glycine digestibility was lower with the control than with the bacteria diet. Except for threonine and alanine, the differences in apparent faecal amino acid digestibility were reduced, although of the same type. Using the apparent digestibility of amino acids, measured for both diets, the values may be computed for a diet containing only bacteria protein. These values are lower than those obtained for the control diet, and the differences evolve in the same way as those observed between the control and the bacteria diets in the ileal digesta and the faeces.

TABLE 6

Quantities of protein and amino acids ingested (g/kg$^{0.75}$/d)
and urea and free amino acid levels in blood (mg per 100 g)

Feed	Control		Bacteria	
	Quantity ingested	Blood level	Quantity ingested	Blood level
Urea	—	11.80	—	27.90
Aspartic acid	1.13	0.53	1.17	0.33
Threonine	0.68	2.27	0.63	1.61
Serine	0.74	1.67	0.58	1.31
Glutamine } Glutamic acid }	3.27	1.32	2.24	1.09
		2.24		1.23
Proline	1.46	1.90	0.93	1.90
Citrulline	—	1.45	—	2.00
Glycine	0.29	3.75	0.49	2.31
Alanine	0.46	2.64	0.71	1.30
Valine	0.92	2.30	0.80	2.83
Cystine	0.15	—	0.11	—
Methionine	0.42	0.66	0.40	0.82
Isoleucine	0.75	1.37	0.64	1.08
Leucine	1.36	1.73	1.12	2.06
Tyrosine	0.72	1.22	0.56	1.61
Phenylalanine	0.72	0.93	0.58	1.00
Ornithine	—	1.07	—	0.95
Lysine	1.14	3.36	1.04	2.86
Histidine	0.38	1.76	0.30	1.56
Arginine	0.51	2.30	0.57	1.20
Protein	14.08	—	13.54	—

In the large intestine, apparent digestibility of the amino acid nitrogen was lower than that of total nitrogen, especially for the control diet. It was particularly depressed for methionine, aromatic amino acids, and in the case of the bacteria diet, for isoleucine. The highest values were found for threonine, serine, glutamic acid, proline, cystine and histidine, as well as for alanine with the bacteria diet.

Apparent digestibility of diaminopimelic acid of the dietary bacteria was negative in the terminal small intestine, and was one of the lowest at the end of the whole digestive tract but the highest in the large intestine.

Blood levels of urea and free amino acids (table 6). — Uremia was higher with the bacteria than with the control diet, especially in one calf where it reached 36.4 mg per 100 g of blood.

The animals fed the control diet ingested more of each essential amino acid, except arginine, than the bacteria calves ; their blood levels of free threonine, isoleucine and histidine were higher than those of the bacteria calves. However, blood concentrations of free valine, methionine, leucine, phenylalanine and tyrosine were lower, while the level of arginine was higher than that of the calves given the bacteria diet. Except for proline and citrulline, non-essential free amino acid levels in the blood of bacteria calves were lower than those of the control animals, although higher amounts of alanine and glycine were ingested.

Discussion.

Although the apparent digestibility of amino acids is difficult to interpret (Mason, 1978), their measurement seems repeatable from one experiment to another. The faecal values obtained for our control diet differed less than 1 p. 100 from those found by Van Weerden and Huisman (1977) and Patureau-Mirand *et al.* (1977) for similar feeds. The results on the amino acid digestibility of our bacteria diet and that used by Van Weerden and Huisman (1977) are similar, if the higher protein level used by those authors (34.3 *vs* 25.5 p. 100 of dry matter) is taken into account by comparing these digestibilities, divided by the digestibility of nitrogen measured in each experiment. In these conditions, the differences are less than 1 p. 100, except in the case of cystine where it is 5.5 p. 100 ; This repeatability of the apparent digestibility of amino acids seems to suggest that the two control diets, as the two bacteria diets, were digested by similar processes in both experiments. However, it does not justify the use of this measurement for describing the protein digestion of a feed.

A. — *Digestion of the control diet.*

In the small intestine. — The amino acid composition of protein in the ileal digesta of the control calves was very different from that of the feed ($\chi^2 = 310$) ; however, this does not prove that there were no dietary amino acids in the digesta, but that no protein had an amino acid composition similar to the mean one of the diet. The digesta protein also had a very different composition from that of pig faecal bacteria (Mason, Just and Bech-Andersen, 1976), used to represent the mean amino acid composition of intestinal bacteria in the preruminant calf since there are no more specific data (table 3). However, the diaminopimelic acid found at that level indicated that gut bacteria protein was present, although in presumably small amounts. The amino acid composition of the ileal digesta was somewhat similar to that of germ-free lamb faeces (Combe, 1976), used to represent the mean composition of the undigested endogenous protein in the preruminant calf. As this protein, the digesta showed a high amount of threonine and a relatively low level of aromatic amino acids. It would appear that the protein recovered in the terminal ileum of preruminant calves contains a large proportion of mucoprotein with those same characteristics. This agrees with the fact that the ileal digesta of milk-fed calves contain a large amount (14 g/16 g N, according to Guilloteau, Patureau-Mirand and Bayle, unpublished data) of amino sugars (glucosamine + galactosamine), and that amount is similar to the level (17 g/16 g N, according to Combe, 1979) found in germ-free lamb faeces. The synthesis of all these observations is obtained by trial and error computation of the proportions of dietary, endogenous and bacterial proteins permitting the best theoretical estimate of digesta amino acid composition ; these proportions are 16, 71 and 13 p. 100, respectively, with a χ^2 of 22 when the computed composition is compared to the measured composition. Digestion of milk protein in the small intestine was almost complete since it appeared that the protein recovered in the terminal ileum of control calves was mainly endogenous.

Digestion in the large intestine. — The amino acid composition of control calf faeces was very different from that of the diet and of germ-free lamb faeces ; it rather resembled the amino acid composition of faecal bacteria, as we have already reported (Patureau-Mirand *et al.*, 1977). The presence of a large proportion of bacteria in the faeces was evidenced by diaminopimelic acid level. Computation showed that the amino acid composition of faecal protein in the control animals could be well estimated by supposing that the faeces contained 74 p. 100 of faecal bacteria protein and 26 p. 100 of ileal digesta protein ($\chi^2 = 19$). The increase in the apparent digestibility of the nitrogen and the amino acids between the terminal ileum and the faeces, already reported in pigs (Holmes *et al.*, 1974 ; Zebrowska, Buraczewska and Buraczewski, 1978) or sheep (Coelho da Silva *et al.*, 1972 ; Ben-Ghedalia *et al.*, 1974), seems to result from the effect of microflora in the caecum and the large intestine on the nitrogen present in the lumen of the terminal ileum (Rérat, 1978 ; Mason, 1978).

B. — *Digestion of the bacteria diet.*

In the small intestine. — The difference in the amino acid composition of the ileal contents in bacteria and control calves suggested a difference in the protein composition, presumably resulting from the presence of dietary bacteria protein at that level. This hypothesis is confirmed by the fact that the additional undigested protein found in the terminal ileum of bacteria calves (in relation to control animals) was particularly rich in diaminopimelic acid and alanine (table 3), and also had a high glutamic acid level. It was thus probably largely constituted by peptidoglycan from the wall of the dietary bacteria. In many gram-negative bacteria that peptidoglycan contains a tetrapeptide having two alanine residues, a diaminopimelic acid residue and a glutamic acid residue (Clarke, Gray and Reaveley, 1967) ; the same has been reported for the wall of dietary bacteria (Richardson and Steer, 1975).

TABLE 7

Quantities of amino acids ingested (In) *and recovered in ileal digesta* (Il) *and faeces* (F) (mg per 100 g of dry matter intake)

Amino acid	Diet		Amino acid	Diet	
	Control	Bacteria		Control	Bacteria
Aspartic acid			Methionine		
— In	2 037	2 211	— In	759	747
— Il	164	232	— Il	21	34
— F	118	163	— F	27	37
Threonine			Isoleucine		
— In	1 227	1 188	— In	1 354	1 211
— Il	170	158	— Il	63	83
— F	60	80	— F	51	76
Serine			Leucine		
— In	1 328	1 084	— In	2 441	2 106
— Il	113	117	— Il	105	144
— F	52	67	— F	79	119
Glutamic acid			Tyrosine		
— In	5 870	4 228	— In	1 290	1 045
— Il	277	340	— Il	56	74
— F	157	184	— F	50	65
Proline			Phenylalanine		
— In	2 631	1 749	— In	1 290	1 096
— Il	134	117	— Il	54	85
— F	49	57	— F	51	74
Glycine			Lysine		
— In	519	926	— In	2 049	1 956
— Il	83	124	— Il	96	126
— F	59	92	— F	73	101
Alanine			Histidine		
— In	835	1 344	— In	683	574
— Il	84	172	— Il	36	46
— F	75	111	— F	20	30
Valine			Arginine		
— In	1 644	1 515	— In	911	1 079
— Il	104	123	— Il	63	85
— F	72	93	— F	52	65
Cystine			Diaminopimelic acid		
— In	266	207	— In	—	54
— Il	64	59	— Il	4	58
— F	35	33	— F	7	8

It is improbable that a large part of that undigested fraction in the small intestine would be derived from intestinal bacteria. The amount of diaminopimelic acid found in the ileum was very similar to that ingested (table 7). Mason and White (1971) also showed in the ruminant sheep that the diaminopimelic acid of the walls of the rumen bacteria is not absorbed, and that the amount provided by small intestine bacteria represents only a small proportion (less than 10 p. 100) of that found in the terminal ileum. Tetrapeptide nitrogen represents only 30 p. 100 of the amino acid nitrogen in the additional undigested protein (including diaminopimelic acid) but in peptidoglycan, the tetrapeptides can be bound by peptidic bridges (Leive, 1973). It is possible that other protein fractions of dietary bacteria were not entirely digested in the small intestine, and that contributed to the amino acid composition of the additional undigested protein. Due to the high value of the apparent nitrogen digestibility measured in the terminal ileum with the bacteria diet, only a small part of the bacterial protein ingested was not digested in the small intestine, although fair amounts appeared in the ileal digesta of bacteria calves.

In the terminal ileum, only serine, threonine, cystine and proline were found in the same amounts with the two diets or in slightly lower quantities with the bacteria diet (table 7). Dietary bacteria threonine was thus absorbed as well as that of milk, the amounts ingested being very similar. This might be true for cystine, serine or proline (although the amounts supplied by the bacteria diet were lower), if the amino acids excreted with the control diet were almost entirely of non-dietary origin. All the other amino acids were excreted in larger amounts with the bacteria diet, no matter what quantities were eaten. Glutamic acid, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and histidine of the dietary bacteria were truly less highly absorbed than those of milk since they were ingested in equal or larger amounts with the control diet. It is difficult to reach a conclusion concerning the other four amino acids (aspartic acid, glycine, alanine, arginine) because the bacteria diet contained higher levels of them.

In the large intestine. — The faecal amino acid and diaminopimelic acid compositions of bacteria calves were similar to those of the control animals ($\chi^2 = 20$). The differences were less than between the ileal digesta ($\chi^2 = 74$) because with both diets the faeces contained mainly faecal bacteria protein. Thus, for the bacteria diet, the mixture of 77 p. 100 of faecal bacteria protein and of 23 p. 100 of the protein recovered in the terminal ileum of bacteria calves had a composition similar to that of the faeces of those animals ($\chi^2 = 8$). The additional undigested protein computed in the faeces was less characteristic than in the ileum, confirming the considerable changes due to the action of large intestine microflora. These changes had two major effects : slight increase of the methionine level and disappearance of a large amount of diaminopimelic acid in the hindgut (table 7). The last observation agrees with the data of Mason and White (1971).

C. — *Digestive and metabolic utilization of dietary bacteria amino acids.*

The concentrations of essential free amino acids in the jugular blood sampled in the first hours after feed intake generally reflect the amino acid composition of the dietary proteins in the preruminant calf (Patureau-Mirand et al., 1974). They can be also used to assess the availability of the dietary amino acids (Pion, 1976) and their metabolic use. The levels of urea and free amino acids in the jugular blood of our calves suggest that some essential amino acids were incompletely converted at sampling in the bacteria calves due to energy deficit. Thus, the percentage of the sum of the essential amino acids in the total free amino acids assayed was higher with the bacteria diet (57 *vs* 52), although intake of most of the essential amino acids was lower than with the control diet. This was confirmed by the relatively low blood alanine level and those of urea and citrulline which were high. The first is strongly catabolized when there is an energy deficit, and the high concentrations of the latter two indicate that some amino acids, especially the non-essential ones, were used for energetic purposes. The low free arginine level in the blood of bacteria calves could result either from a loss of that amino acid during sample preparation or from a high hepatic arginase activity, in bacteria animals, associated with active ureogenesis relative to the energetic utilization of amino acids. This could be explained by the fact that a calf, which did not regularly eat all its allowance, was receiving the bacteria diet at blood sampling. A deficit of dietary sulfur amino acids and/or histidine, shown by their low levels in the blood, might explain the accumulation of some essential amino acids in the blood. However, there was no marked unavailability of any essential amino acid of bacterial protein when postprandial free amino acid levels were studied.

In conclusion, the digestibility of methanol-grown bacteria amino acids is slightly lower than that of milk, both in the terminal small intestine and in the faeces. Digestion of milk and bacterial amino acids (except diaminopimelic acid) takes place almost exclusively in the small intestine. However, due to the considerable changes caused by large intestine flora, a study of ileal digesta protein composition is more useful than a study of faecal protein for estimating the origin of unabsorbed protein. Nevertheless, the effect of the flora on the amino acid composition of ileal digesta is not negligible. The lower digestibility of bacterial protein seems mostly due to the resistance of the protein (mainly peptidoglycan) in the bacterial wall to digestion in the small intestine. However, the differences observed are small as compared to milk protein, and do not indicate the unavailability of any particular essential amino acid either at the digestive or the metabolic level.

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Résumé. Le but de cet essai était d'étudier le bilan de la digestion des acides aminés du lait et des bactéries cultivées sur méthanol à la fin de l'intestin grêle et à celle du tube digestif du veau préruminant. Deux aliments d'allaitement (Témoin et Bactéries) ont été utilisés. Les matières azotées de l'aliment Témoin étaient fournies exclusivement par la poudre de lait écrémé ; 50,5 p. 100 de celles de l'aliment Bactéries étaient apportées par des bactéries cultivées sur méthanol et le reste par la poudre de lait écrémé et des acides aminés de synthèse.

La digestibilité apparente de tous les acides aminés dosés, méthionine exceptée, est moins élevée à la fin de l'intestin grêle que dans l'ensemble du tube digestif, mais les

différences observées sont généralement plus faibles que pour l'azote total (tabl. 5). L'écart le plus important concerne la cystine dont la digestibilité apparente passe de 76 p. 100 à la fin de l'iléon à 87 p. 100 au niveau des fèces pour l'aliment Témoin, les valeurs correspondantes pour les bactéries alimentaires étant de 64 et 79 p. 100. Après la cystine, ce sont la thréonine du lait et l'acide glutamique des bactéries alimentaires dont les quantités apparemment utilisables par les veaux sont les moins bien appréciées par les mesures au niveau des fèces. L'acide diaminopimélique des bactéries alimentaires ne semble pas absorbé dans l'intestin grêle mais disparaît en très grande partie dans le gros intestin (tabl. 7). La digestibilité des acides aminés, sauf celle de la thréonine et de la glycine à la fin de l'iléon ainsi que celle de la glycine et de l'alanine au niveau des fèces, est plus élevée pour le lait que pour les bactéries alimentaires (tabl. 5).

Les digesta iléaux sont plus riches que les fèces en thréonine, sérine, proline et cystine et sont moins pourvus en méthionine, isoleucine, leucine, tyrosine, phénylalanine, lysine et arginine (tabl. 2 et 3). Ces différences peuvent résulter d'une plus grande proportion de protéines endogènes dans les digesta et d'une moindre quantité de protéines de bactéries du tube digestif. Les digesta contiennent également davantage de protéines alimentaires que les fèces, au moins dans le cas de l'aliment Bactéries, comme l'indiquent les teneurs en acide diaminopimélique observées avec ce régime. Les proportions respectives de protéines d'origine alimentaire, endogène et microbienne estimées à la fin de l'iléon sont de 17, 72 et 11 p. 100 avec l'aliment Témoin.

La digestibilité moins élevée des matières azotées des bactéries cultivées sur méthanol semble due en grande partie à la résistance des protides de leur paroi, notamment du peptidoglycane, à la digestion dans l'intestin grêle. Toutefois, les différences observées par rapport aux protéines du lait sont faibles ; elles ne permettent pas de mettre en évidence l'indisponibilité d'un acide aminé particulier, acide diaminopimélique excepté, au niveau digestif ou métabolique.

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