Bacterial D-alanine concentrations as a marker of bacterial nitrogen in the gastrointestinal tract of pigs and cows

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ABSTRACT: D-alanine (DAL) has been successfully used as a marker of bacterial nitrogen (N) in the small intestine of cows. This study compares DAL contents of intestinal bacteria in digesta of cows and pigs with respect to diet and sampling site. In isolated ileal bacteria of pigs a DAL/N ratio (41.72 ± 3.19 mg/g, n = 18) was found, which was not different from that in rumen bacteria (40.11 ± 1.95 mg/g, n = 18) but higher than in duodenal bacteria of cows (38.09 ± 2.09 mg/g, n = 18, P < 0.001). The DAL/N ratio in ileal bacteria of pigs was independent of the diet (P = 0.38) but it tended to be affected by the animal (P = 0.095). In bacterial preparations derived from cows, the DAL/N ratio depended on the diet (P = 0.04) and the site of sampling (P = 0.004). Our findings indicate that a general value for DAL/N ratio in pig or cow intestinal contents to calculate bacterial N should not be used.

Keywords: D-alanine; bacterial nitrogen; marker concentration; intestine; pig; cow

The knowledge of the proportion of bacterial nitrogen (N) to total N in intestinal effluents of mammals is necessary to quantify bacterial N in the small intestine. For this purpose, specific bacterial markers are used. This approach is only feasible if the marker to N ratio in the bacterial mass is known and the bacterial sample isolated for determination of marker to N ratio is representative for the bacterial fraction in the digesta.

Methods for tracing the bacterial protein include labelling of protein with ^{15}N (Siddons et al., 1985; Firkins et al., 1992), ^{35}S (Whitelaw et al., 1984) and ^{32}P (Van Nevel and Demeyer, 1977). Also the use of substances which are present as constitutive components in bacteria, e.g. diaminopimelic acid (DAPA) (Czerkawski, 1974; Masson et al., 1991) or RNA (Ling and Buttery; 1978; Schoehusen et al., 1990) has been reported. Garrett et al. (1987), Ueda et al. (1989) and Caspo et al. (2001) suggested that D-amino acids such as D-alanine (DAL) and D-glutamic acid are suitable as chemical markers for bacterial DM and N. D-amino acids are commonly found in the peptidoglycan-polysaccaride complexes of the bacterial cell wall (Schleifer and Kandler, 1967; Trippen et al., 1979). DAL has the advantage over other markers of being present in both Gram-positive and Gram-negative bacterial cell walls (Schleifer and Kandler, 1967). However, the concentration of DAL in cells varies according to the cell type and the amount of peptidoglycan in cells (Quigley and Schwab, 1988), a disadvantage to its use as bacterial marker (Ling and Buttery, 1978) In our previous study on protein synthesis in the rumen of cows DAL, DAPA, RNA, ^{15}N, and amino acid (AA) profile have been compared as bacterial markers (Schoenhusen et al., 1995). We found

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that the reproducibility was superior with the DAL marker followed by ^{15}N labelling, and the results obtained with DAL and ^{15}N agree closely within the same source of bacteria. The low coefficient of variation in the DAL to N ratio in the bacterial biomass and the DAL specificity to bacteria indicate that DAL is superior to many other bacterial markers currently used. DAL was detected only in feeds and foodstuffs which undergo bacterial fermentation (Voss and Galensa, 2000).

There have been no studies on the evaluation of DAL as indicator of bacterial N in intestinal digesta of monogastric animals. The estimation of the proportion of bacterial N in small intestine of pigs is important for the evaluation of basal endogenous N secretions. Ileal endogenous N and AA excretion is of practical importance for the evaluation of dietary proteins, in terms of the amount of precaecal digestible AA. Therefore, we measured the DAL content as a marker for bacterial proteins in ileal digesta of pigs fed various diets differing in ileal digestible lysine and crude protein to provide data on the variability of this indicator related to the diet. Further, in order to determine possible species dependent differences in the bacterial DAL content we compared DAL content of porcine and bovine bacteria.

MATERIAL AND METHODS

Animals, diets and sampling

The study was carried out in pigs and cows. Details concerning pigs used in the present experiment, diets and experimental design were described by Hennig et al. (2004). Briefly, three growing Saddleback pigs (mean body weight (BW) at start 27 ± 4 kg, at the end 59 ± 4 kg) fitted with an end-to-end-ileorectal anastomoses conserving the ileocecal valve (Hennig et al., 1990; Laplace et al., 1994) were fed six diets differing in ileal digestible lysine and crude protein (CP) (Table 1). Three diets were based on barley, rye and oilseed by-products and three diets consisted of wheat and milk powder. Every trial period consisted of seven days. The first four days were used for adaptation and the following (last) three days for collection of ileal digesta. Digesta samples (n = 18) were quantitatively collected in a mixture (99.5:0.5; vol/vol) of ethanol (95 vol%) and formaldehyde (28% wt/vol) to abolish microbial activity, homogenized and stored at 4°C until bacteria isolation.

Three dairy cows (German Holstein, mean BW 602 ± 27 kg), each fitted with a rumen cannulae (Bergfeld et al., 1968) and a re-entrant cannulae in the duodenum (about 20 cm beyond the ductus choledochus and the ductus pancreaticus, Engelmann et al., 1990), received six isonitrogenous and isoenergetic diets (mean intake of 15.0 ± 0.50 kg dry matter (DM)/day; forage:concentrate ratio = 50:50) in a periodic experiment. The cows were housed in metabolism cages with free access to water. Diets were offered twice daily (05.50 and 14.50 h) in equal parts. Diets were composed of grass and maize silage, hay, dried feed rye, dried clover, beet pulp, a grain mixture and protein supplements differed in their rates of ruminal protein degradability. Diets were fed without and with supplementation of a yeast culture (Table 2). Yeast cultures were used to modify the bacterial pattern in the rumen (Erasmus et al., 1992). Each

Table 1. Nutrient content of barley and oilseed meals (Diet 1, 2, 3) and wheat and milk powder based diets (Diet 4, 5, 6) (DM basis) fed to pigs (Hennig et al., 2004)

	Diet (growth periods)					
	1 (E)	2 (M)	3 (F)	4 (E)	5 (M)	6 (F)
CP (g/kg)	180	170	149	203	184	167
ME (MJ/kg)	14.9	15.1	14.9	15.3	15.2	15.0
Calculated apparent ileal digestible lysine content[1] (g/kg)	6.4	6.2	5.3	6.1	6.1	5.8

E, M and F = early, middle and finishing growth periods

ME = metabolizable energy

[1]based on total lysine concentration of each component multiplied by digestibility coefficients for lysine (Hennig et al., 1990)

experimental period lasted three weeks. After an adaptation period of 12 days, rumen fluid (2 l) was collected 3.5 h after the morning feeding. Rumen bacteria (n = 18) were harvested immediately after rumen fluid sampling. Duodenal samples (300 ml) were collected over a 24-h period at 6-h intervals. Duodenal samples (n = 18) were kept at 4°C till isolation of the bacterial mass.

Isolation of bacterial mass

Bacterial mass from ileal digesta of pigs was isolated by fractionated centrifugation as previously outlined by Stephen and Cummings (1980) with a modification of Meinl and Kreienbring (1985). The first step of isolation consisted of filtering the digesta sample (37°C) through a gauze (50 µm) and centrifugation of the filtrate at 340 × g, 10 min at 25°C. This pellet was washed three times with a mixture of ethanol and formaldehyde (99.5:0.5; vol/vol), (100 ml conservation solution for 200 g chyme) and separated from the filtrate. The combined filtrates were centrifuged once more at 340 × g, 10 min at 4°C. From the supernatant, the bacterial fraction was isolated by a centrifugation at 22 000 × g, 45 min at 4°C. This pellet was washed with saline (0.9% NaCl, wt/vol) and iso-propanol, the supernatant washing fluid was separated by centrifugation at 22 000 × g, 45 min at 4°C. The isolated bacteria were dried by lyophilisation.

Bacteria from rumen fluid and duodenal digesta of cows were isolated by a two-stage centrifugation procedure as described by Krawielitzki and Voigt (1988). Briefly, after filtering the sample through gauze, washing the residue with physiological saline solution, the filtrate was centrifuged at 550 × g, 15 min at 4°C to remove protozoa and food particles. The bacterial fraction was isolated by a second centrifugation at 30 000 × g, 45 min at 4°C. This pellet was washed twice by dispersing in a physiological saline solution and iso-propanol. The suspensions were each time centrifuged at 30 000 × g 10 min at 4°C. The isolated bacteria were dried by lyophilisation.

Batch cultures at 37°C for 5 days were performed with the selected bacteria known to be important members of porcine intestinal digesta (Table 3). A *Lactobacillus plantarum* strain from a commercial silage additive and another *Lactobacillus* sp., isolated from the ileal digesta of pigs were cultured using MRS-broth (De Man et al., 1960). *Enterococcus faecium* (DSM 5464) from a probiotic piglet gel and another *Enterococcus* sp., isolated from the pig ileal digesta were cultured using Enterococci selective broth without agar according to the medium of Slanetz and Bartley (1957). A non-specified strain belongs to the family of Enterobacteriaceae, was isolated from ileal digesta of pigs using Violet-red Bile Dextrose (VRBD)-agar (Mossel et al., 1962; Mossel and Cornelissen, 1963). Bacterial cells were harvested and washed three times with sterile saline (0.9% NaCl, wt/vol) by centrifugation (25 400 × g, 45 min, 20°C).After their isolation bacterial mass was dried by lyophilisation.

Analytical procedures

DM of bacterial samples was determined according to the 'Weende' procedure (Naumann and Bassler, 1997). The N content of ileal bacteria of pigs and pure bacterial cultures was analyzed applying the Dumas-combustion method using a LECO, CNS-2000 (Leco Instrumente GmbH, Mönchengladbach, Germany). The N content of rumen and duodenal bacteria of cows was estimated by the standard Kjeldahl procedure (AOAC, 2001).

Samples for DAL analysis were hydrolyzed in an atmosphere of CO_2 by addition of 6M HCl to 200 mg bacterial mass or 600 mg milled foodstuff (1 ml HCl/mg N) in a drying oven for 22 h at 110°C. After adding of activated charcoal (0.1 g) and mixing thoroughly, hydrolysed samples were filtered (589 blue ribbon, Schleicher & Schuell, Dassel, Germany) and evaporated at 40°C to dryness in a rotary evaporator (Laborota 4000, Heidolph Instruments, Schwabach, Germany). The residues were re-suspended two times in distilled water (15 ml, 80°C), and evaporated to dryness. Finally, they were re-suspended with 0.1M HCl to a final volume of 10 ml. DAL content of the bacterial HCl-hydrolysate was determined in triplicate as outlined by Garret et al. (1987). Briefly, 100 µl of re-suspended hydrolysed sample were mixed with 11 µl D-amino acid oxidase (20 IU/ml, Sigma, St. Louis, USA), 5 µl FAD (1 mg/ml, Sigma), 10 µl catalase (0.5 g/ml, Sigma) and 1.58 ml sodium pyrophosphate buffer (0.1M, pH 8.3, oxygenated; Merck KGaA, Darmstadt, Germany). This mixture was incubated for 2 h at 39°C in an atmosphere of O_2. A 1-ml aliquot of oxidase reaction solution, 1.96 ml triethanolamine (0.2 mol/l) EDTA buffer

(pH 7.6) and 30 µl NADH (10 mg/ml) were added. After equilibration for 3 min the optical density of the solution was read in a 1-cm silica glass cuvette at 340 nm (Ultrospec 2000, Amersham Pharmacia Biotech, Cambridge, UK). Then, 12 µl LDH (6 025 IU/ml, Sigma) were added to the solution, and after a reaction time of 3 min the final optical density was read. The amount of DAL formed in experimental samples from racemization during hydrolysis, basically during acidic treatment, was regarded as less important. As shown by Ueda et al. (1989), lowering the hydrolysis temperature from 150°C to 105°C reduces the degree of racemization substantially. Calibration was done with dilutions of a non-hydrolysed DAL standard stock solution (89 µg DAL/ml H_20, Sigma).

The AA content of bacterial samples was determined by liquid ion-exchange chromatography (Biochrom 20, Pharmacia LKB Biochrom Ltd., Cambridge, UK) following 6M HCl hydrolysis (22 h, 110°C) except for tryptophan which involved 4M NaOH hydrolysis (26 h, 110°C). Cysteine and methionine were determined as cysteic acid and methionine sulfone after oxidation with performic acid (85% wt/vol, 16 h, 0°C) followed by 6M HCl hydrolysis (22 h, 110°C). No corrections were made for losses of AA during hydrolysis.

Statistical analysis

Data are given as means with their standard deviation (SD). Results were statistically analysed by the software programme Statistica for the Windows™ operating system (StatSoft Inc. Version 6.0, 1994). Within experiments in pigs and cows, respectively, DAL to total N ratios of isolated bacteria were analysed by one-way ANOVA using animal (pig, cow), diet, (pig, cow), sampling site of bacteria (only cow) and diet × sampling site interaction (only cow) as main effects. Differences were localised by LSD *t*-test. Differences of bacterial sources (ileal digesta of pigs, rumen and duodenal digesta of cows) on total N, DAL, DAL to total N ratios, AA-N, total AA, and proportions of AA in isolated bacteria were analysed by one-way ANOVA. If the *F*-test showed significance ($P < 0.05$), the differences were evaluated with LSD *t*-test.

Table 2. Composition (kg DM/animal and day) and nutrient content of diets without (Diet 1, 2, 3) and with supplementation of a yeast culture (Diet 4, 5, 6) fed to dairy cows

	Diet					
	1	2	3	4	5	6
Grass silage	3.6	3.6	2.9	3.3	3.6	2.9
Maize silage	2.1	2.1	1.4	2.1	2.1	1.4
Grass hay	0.7	0.7	1.3	0.7	0.7	1.3
Dried feed rye	0.6	0.6	0.6	0.6	0.6	0.6
Dried red clover	–	–	2.0	–	–	2.0
Dried beet pulp	1.3	1.3	1.3	1.3	1.3	1.3
Grain mixture (80 barley, 20 wheat)	5.5	5.5	4.6	5.5	5.5	4.6
Urea	0.009	0.009	0.009	0.009	0.009	0.009
Soybean meal	0.8	–	–	0.8	–	–
Protected protein concentrate	–	0.8	–	–	0.8	–
Pea meal	–	–	1.3	–	–	1.3
Live yeast culture (*Saccharomyces cerevisiae brewery*)	–	–	–	0.02	0.02	0.02
Crude protein (g/kg DM)	156	159	159	154	156	154
Crude fibre (g/kg DM)	172	170	179	170	174	172
Net energy fat (MJ/day)	90.1	90.8	95.2	91.0	91.2	95.9

RESULTS

The DAL/N ratio in isolated ileal bacteria of pigs was independent of the diet ($P = 0.38$) but it tended to be affected by the individual animal ($P = 0.095$) (not shown). In bacterial preparations derived from cows, the DAL/N ratio depended on the diet ($P = 0.038$) and the site of sampling ($P = 0.004$), but there was no significant diet × sampling site interaction. The coefficients of variation for the DAL/N ratio were 7.0 and 4.8% for samples from pigs and cows, respectively (not shown).

The mean DAL/N ratio (mg/g) in ileal bacteria of pigs was not different to that in rumen bacteria, but both bacterial DAL/N ratios were higher ($P < 0.001$) than in duodenal microbes of cows (Table 4). The bacterial DAL proportion found in ileal bacterial protein of pigs was higher than in the rumen ($P < 0.001$) and the duodenal ($P < 0.001$) bacterial protein of cows. The concentrations of total N and DAL in bacteria collected from the pig ileum were lower than in bacteria harvested from the rumen and duodenum of cows ($P < 0.001$). The contents of N and DAL were closely related in ileal bacteria of pigs ($r = 0.96$) and rumen bacteria ($r = 0.91$), but less in duodenal bacteria of cows ($r = 0.55$) (not shown).

Total AA and AA-N were lowest ($P < 0.01$) in bacteria of pigs and were lower ($P < 0.05$) in bacteria of duodenum than rumen in cows (Table 4). Ileal bacterial protein of pigs in general contained higher proportions of Arg, His, Leu, Trp, Val, Cys, Ala, Glu, Pro and Ser ($P < 0.05$) and smaller of Ile, Lys, Met and Tyr ($P < 0.05$) than bacterial protein from the rumen and duodenum. Proportion of Gly in bacterial protein was lower ($P < 0.05$) in ileum of pigs than in duodenal of cows. Differences in the AA pattern between rumen and duodenal bacterial protein were present for Lys, Trp, Cys, Asp and Gly ($P < 0.05$).

DISCUSSION

In view of the absence or the relative low concentrations of DAL in specific foodstuffs and the selective retention in the digestive tract, the contribution of feed-DAL to the total amount of DAL in the small intestine seems to be of little quantitative importance (Greife et al., 1985). Among the feeds used for pigs in this study DAL was not detected in barley, wheat, rye and soy bean. Only in skim milk powder, measurable quantities of DAL (0.02 mg/g DM) are present (Brueckner and Hausch, 1990; Albertini et al., 1996). As expected, DAL was detected in silage (0.6 mg/g DM), but not in grain and protein concentrates fed to cows. Therefore, it can be assumed that intestinal DAL is largely specific for protein of bacterial origin.

In a preliminary study we determined the DAL content of pure bacterial cultures known to be important members of porcine intestinal digesta to evaluate the DAL/N ratios in individual bacterial cultures (Schoenhusen et al., unpublished observations). The DAL/N ratio in mixed bacteria harvested from the ileal chyme of pigs and from the rumen and duodenum of cows as well as in the gram positive *Lactobacilli* and *Enterococci* were similar, but

Table 3. Ratio of D-alanine/N (mg/g), concentrations of total N (g/100 g DM), AA-N and total AA (g/16 g N), and proportions of alanine and D-alanine (g/100 g total analysed AA) in pure bacterial cultures of *Lactobacilli*[1], *Enterococci*[2] and Enterobacteriaceae[2]

	Bacterial strain/family				
	Lactobacillus plantarum	*Lactobacillus* ssp.	*Enterococcus* sp.	*Enterococcus faecium*	Entero-bacteriaceae
D-Alanine/N	40.21	39.47	39.07	37.46	10.40
Total N	11.04	10.90	10.63	7.75	13.71
AA-N	8.42	8.82	10.62	12.92	9.94
Total AA	61.00	64.63	77.33	94.61	70.99
Alanine	14.31	19.51	10.89	7.16	9.00
D-Alanine	1.05	0.98	0.79	0.63	0.23

[1]using Anaerocult A (Merck KGaA, Darmstadt, Germany) to provide anaerobic conditions in anaerobic jars
[2]aerobic conditions

Table 4. Ratio of D-alanine/N (mg/g), concentrations of total N (g/100 g DM), AA-N and total AA (g/16 g N), and proportions of AA (g/100 g total analysed AA) in bacterial fractions collected from ileal digesta of pigs, from rumen and duodenal digesta of cows (data were presented as means ± SD)

	Source of bacteria			Effect of source of bacteria *P*-value
	pig ileum (n = 18)	cow rumen (n = 18)	cow duodenum (n = 18)	
D-Alanine/N	41.72 ± 3.19[a]	40.11 ± 1.95[a]	38.72 ± 3.19[b]	< 0.001
Total N	5.83 ± 1.64[a]	8.31 ± 0.59[b]	8.54 ± 0.28[b]	< 0.001
AA-N	9.41 ± 0.68[a]	12.60 ± 0.51[b]	12.17 ± 0.45[c]	< 0.001
Total AA	69.06 ± 4.88[a]	93.85 ± 4.04[b]	90.15 ± 3.83[c]	< 0.001
Alanine	8.10 ± 0.85[a]	6.59 ± 0.39[b]	6.89 ± 0.29[b]	< 0.001
D-Alanine	0.97 ± 0.09[a]	0.69 ± 0.05[b]	0.67 ± 0.04[b]	< 0.001
Arginine	5.24 ± 0.32[a]	4.92 ± 0.25[b]	4.87 ± 0.31[b]	< 0.001
Histidine	2.08 ± 0.09[a]	1.68 ± 0.16[b]	1.76 ± 0.12[b]	< 0.001
Isoleucine	4.57 ± 0.28[a]	5.78 ± 0.23[b]	5.79 ± 0.35[b]	< 0.001
Leucine	8.93 ± 0.47[a]	8.32 ± 0.44[b]	7.38 ± 2.72[b]	< 0.001
Lysine	4.81 ± 0.43[a]	7.98 ± 0.29[b]	7.62 ± 0.42[c]	< 0.001
Methionine	1.59 ± 0.14[a]	2.51 ± 0.18[b]	2.56 ± 0.24[b]	< 0.001
Phenylalanine	5.65 ± 0.96	5.62 ± 0.24	5.53 ± 0.33	< 0.83
Tyrosine	0.84 ± 0.19[a]	4.28 ± 0.17[b]	4.35 ± 0.18[b]	< 0.001
Threonine	4.82 ± 0.22	4.92 ± 0.10	4.86 ± 0.18	< 0.20
Tryptophan	1.51 ± 0.14[a]	0.59 ± 0.19[b]	0.89 ± 0.17[c]	< 0.001
Valine	7.02 ± 0.22[a]	5.99 ± 0.24[b]	6.14 ± 0.25[b]	< 0.001
Cysteine	2.01 ± 0.23[a]	1.47 ± 0.14[b]	1.77 ± 0.14[c]	< 0.001
Aspartic acid	12.26 ± 0.51[ab]	12.39 ± 0.25[b]	12.03 ± 0.46[a]	< 0.04
Glutamic acid	13.98 ± 1.10[a]	13.06 ± 0.28[b]	12.73 ± 0.41[b]	< 0.001
Glycine	5.38 ± 0.24[a]	5.28 ± 0.15[a]	6.21 ± 0.33[b]	< 0.001
Proline	5.26 ± 0.58[a]	4.18 ± 0.59[b]	4.08 ± 0.42[b]	< 0.001
Serine	5.95 ± 0.44[a]	4.44 ± 0.14[b]	4.54 ± 0.18[b]	< 0.001

[a, b, c]mean values within a row with different letters are significantly different ($P < 0.05$)

contrasted strikingly with the used strain belongs to the family of gram negative Enterobateriaceae (Table 3). *Lactobacilli* were a dominant group (9 log colony-forming units (cfu)/g effluent) in the ileum of pigs, whereas microbial counts of *Enterococci* (6 log cfu/g effluent) and Enterobacteriaceae (6 cfu/g effluent, unpublished data) were numerically lower (Hennig et al., 2004), and *Lactobacillus plantarum* and *Enterococcus faecium* belong to the normal intestinal microbiota of pigs (Bates et al., 1985; Du Toit et al., 2001). Gram-negative bacteria represent also a considerable proportion of bacteria in ruminal fluid and intestinal digesta (Hungate, 1966). DAL can be found in all bacteria containing peptidoglycan, but the peptidoglycan content varies according to the cell type. Gram-positive bacteria contain about 30–60% peptidoglycan in the cell wall, and thus show a higher concentration of D-alanine, whereas gram-negative bacteria, such as Enterobacteriaceae have only 10% peptidoglycan (Schleifer and Kandler, 1967; Fukuhara et al., 1983). This is in line with the present finding that Enterobacteriaceae contain only a third of the DAL/N content as compared to *Lactobacilli*

and *Enterococci* (Table 3). In pigs, 12% of the total alanine in ileal bacterial protein were present as D-isomer. This portion was in the same range for rumen and duodenal bacterial protein of cows (10%). The contribution of DAL to total alanine was in the range of 5–9% in *Lactobacilli* and *Enterococci*, but it was only 2.5% in Enterobacteriaceae, which suggests that the bovine and porcine bacterial isolates investigated here rather consist of gram-positive bacteria. Studies on the AA composition and sequence of peptidoglycans of different Gram-negative bacteria have shown that there is no great variation within this group. However, the Gram-positive bacteria reveal, contrary to the Gram-negative organisms, a great variation in the composition and structure arrangement of their peptidoglycans (Schleifer and Kandler, 1972).

In pigs, we did not find a dietary effect on the DAL/N ratio in the ileal digesta which is in line with the observation that the intestinal microbiota of pigs was not altered by the diet (Hennig et al., 2004). In cows, dietary effects on the DAL/N ratio were possibly related to changes in colonization (Yang, 1991) and composition of the rumen bacteria (Garrett et al., 1987). In addition, variations in the physical state of the bacterial cells were associated with changes in growth and turnover rates, which can alter their cell wall/protoplasma ratio (Cecava et al., 1990). In ruminants, dietary factors have been noticed to induce differences in the ratio of Gram-negative to Gram-positive bacteria (Hungate, 1966), as well in the distribution and chemical composition of liquid and solid-associated bacterial populations in the rumen of sheep and dairy cows (Rodriguez et al., 2000; Yang et al., 2001). Alterations in the microbes attributed to cell lyses and proteolysis may be responsible for observed sampling site effects (Mathers and Miller, 1980). Rumen bacteria were isolated from a fresh sample withdrawn from the rumen 3.5 h after feeding, whereas duodenal bacterial fraction was isolated from a sample of digesta which was taken over 24 h. Differences in the DAL/N ratio between bacterial isolates from rumen and duodenum can be, in addition, attributed to the fact that either the rumen bacteria are not representative for the bacterial population in the rumen and/or in the omasum a microbial activity is present (Giesecke and von Engelhard, 1975). The technique we used for bacterial isolation harvest free-floating bacteria from the liquid phase of digesta. Similar DAL concentrations for rumen bacteria in young calves (44.5 mg/g N) have been reported by Quigley and Schwab (1988), and the average N content was 8.2% of DM. Garrett et al. (1987) reported higher amounts of 64.2 mg DAL/g N in rumen bacteria isolated from mature cattle, with total N in bacterial isolates account for 5.84% of DM. Greife et al. (1985) reported only 24.2 mg DAL/g bacterial N in cows. These authors suggest that variations of the DAL/N ratios may be caused by the digesta sampling procedure and the method of bacterial isolation. Unfortunately, animal and dietary effects on DAL to N ratio were not investigated in these studies.

The bacterial protein from the ileum of pigs showed a smaller amount of total AA than bacterial protein from the rumen and duodenum of cows. This reflects the fact that pig ileal bacterial-N contains a higher proportion of non-AA-N. This difference could be partially associated with a higher RNA-N concentration observed in ileal bacteria. In ileal bacteria of pigs we have found 2.01 ± 0.34 g RNA-N/16 g N that was significantly higher than in rumen (1.55 ± 0.07 g RNA-N/16 g N) and duodenal bacteria (1.60 ± 0.16 g RNA-N/16 g N) of cows (Schoenhusen et al., unpublished observations). The RNA-N content in *Lactobacilli* were similar (2.09 g RNA-N/16 g N) as in ileal bacterial protein of pigs. Bacterial preparations were not, as might have been expected, consistent in their cytoplasmic material (Bates et al., 1985; Meinl and Kreienbring 1985).

The results show that DAL as marker can be used equally well as in the case of the cattle for estimation of bacterial protein in intestine. However, because diet and animal specific effects as well as the sampling site affects the DAL contents, a general value for bacterial DAL/N ratio in pig or cow intestinal contents should not be used. Thus, it is necessary to determine the DAL concentration in isolated bacteria of each individually fed animal.

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