Quantitation by hybridization probes of *Lactobacillus delbrueckii* in the terminal ileum of fistulated Göttingen miniature pigs after feeding of yoghurt

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1. Introduction

The intestine of vertebrate animals is a complex ecosystem which is estimated to contain several hundreds of species of predominantly anaerobic microorganisms. This microflora is implicated in both beneficial and detrimental effects on health and well-being of the host. Lactic acid bacteria are natural inhabitants of the intestinal tract. They are also used as fermentative organisms that effect flavour, texture and preservative qualities of most fermented foods. Lactobacilli and bifidobacteria are the most important groups that are considered to bring forth probiotic strains owing to their roles as members of the indigenous microflora of humans. Phylogenetic studies have recognized 54 species of lactobacilli, 18 of which are regarded to enclose strains of some interest for probiotic products, and 31 species of bifidobacteria, 11 of which have been detected in human faeces (1, 2). Members of the species *L. delbrueckii* are used for the fermentation of several milk products, e.g. the subspecies *bulgaricus* for the manufacture of yoghurt. The subspecies *lactis,* however, has been found as a normal inhabitant of the mammalian intestinal tract (3).

Since there is still confusion about identity, viability, survival, and activity of potential probiotic strains when ingested together with food, we intended to analyse detectability and survival (4) upon ingestion of one of the starter bacteria contained in yoghurt. We compared the detectability of *L. delbrueckii* in the digesta of two minipigs after uptake of a semisynthetic diet and of heat-treated yoghurt with that of living *L. delbrueckii* in yoghurt.

We chose minipigs for our studies as they are one of the best animal models due to their omnivorous behaviour and their digestive tract being close to that of man. Fistulas were implanted at the terminal ileum just in front of the entrance to the large bowel. This allowed analysis of an otherwise hardly accessible part of the digestive tract.

In this work, we measured the proportion of *L. delbrueckii* DNA by hybridization of total DNA extracted from a series of chyme samples, and we correlated it with *L. delbrueckii* counts (colony forming units) of a standardized pure culture. We estimated total eubacterial DNA and assessed the proportion of *L. delbrueckii* in relation to the eubacterial DNA between 3 and 7 hours postprandially. However, the design of our experiments did not allow to distinguish between the ingested *L. delbrueckii* subsp. *bulgaricus* and the endogenous *lactis* DNA. This differentiation has been carried out in another study (4)

where we additionally demonstrated that a remarkable proportion of both starters (*L. delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*) had survived the gastrointestinal passage up to the site of the fistula.

2. Material and Methods

2.1 Bacterial strains

Strains used were from the collection of the Institute of Microbiology of the Federal Dairy Research Center in Kiel, FRG. Lactobacilli were grown on MRS agar (6) and streptococci on a modified M17 agar (7).

2.2 Animals

Göttingen miniature pigs, bred specific pathogen free (8), were fitted with T-cannulas at the terminal ileum. All experimental procedures described followed the guidelines for the care and use of laboratory animals and were approved by the Animal Care and Animal Ethics Committee of the Ministry of Environment of Schleswig-Holstein, FRG.

About one year after implementation of the fistulas feeding experiments were carried out with two male minipigs (mean body weight 30 kg). During the experiments they were housed individually in metabolic cages. Four weeks before starting the experiments they were exclusively fed a semi-synthetic diet.

2.3 Yoghurt manufacture

UH-treated milk was heated to 100°C for 10 min and was allowed to cool down to about 40°C. It was immediately inoculated with a mixture of *L. delbrueckii* subsp. *bulgaricus* Kt4 and *S. thermophilus* 71 and incubated at that temperature until the yoghurt was set. Heat-treated yoghurt was prepared by heating for 30 min at 100°C to inactivate the starter cultures.

2.4 Feeding experiments with fistulated Göttingen miniature pigs

The feeding trials were carried out on three subsequent days and were repeated one week later. After an overnight fast, the animals received one single meal in the morning. A semisynthetic diet was fed on day 1 (Sacas 15 consisting of margarine 7.5%, lard 7.5%, cellulose 6.0%, vitamins and minerals 8.0%, corn starch 29.0%, sucrose 24%, casein 15%, lacty ® (9) 3%). On day 2, heat-treated yoghurt was fed: 200 g in the first week and 400 g in the second week. On day 3, 400 g of yoghurt containing living *L. delbrueckii* subsp. *bulgaricus* at 2×10^9 cfu/g in the first week and 1×10^8 cfu/g in the second week were applied. 2% $Cr^{III}_2O_3$ was added as a gastrointestinal transition marker to the yoghurt in the second week. Water was offered *ad libitum*. The intestinal tract was temporarily closed with a balloon catheter. Samples were collected in balloons attached to the fistulas as the digesta passed through the intestine. Balloons were changed approximately every hour depending on the amount of samples that had flown out. The sampling times are described in respective experiments (see Fig. 1). Digesta collected were immediately frozen in liquid nitrogen and stored at –72 °C.

2.5 DNA extraction

0.5 g of chyme were weighed and total DNA was prepared according to Leenhouts (10) and dissolved in 250 µl TE-buffer (Tris 10 mM, EDTA 1 mM, pH 8.0).

2.6 Dot blot hybridization

10, 20, and 50 µl of DNA solution extracted from each chyme sample were denatured in 250 µl NaOH (0.4 N) for 30 min before they were spotted onto a positively charged nylon membrane using a Schleicher & Schüll minifold apparatus (Dassel, FRG). As positive control, DNA was extracted from *L. delbrueckii* subsp. *bulgaricus* Kt4 propagated in MRS medium to an optical density (OD_{620}) of 0.7 or 1.0 and dissolved in the same volume of TE-buffer as the original culture. 10, 20, and 50 µl thereof were denatured (see above) and also spotted onto the membrane. (Pre-)hybridization solution was 6 × SSPE (20 × SSPE: 3.6 M NaCl, 0.4 M NaH_2PO_4, 0.02 M pH 8.0 EDTA), 5 × Denhardt: 2% Ficoll 400 (w/v); 2% (w/v) polyvinylpyrrolidon, 2 % BSA (w/v), 0.5 % SDS, and 100 mg sheared herring sperm DNA. Labelling of the *L. delbrueckii*-specific probe (11) was carried out with α-^{32}P-dATP and T_7-polymerase in a random priming reaction as described by the manufacturer ("Prime it", Stratagene, La Jolla, Ca.). Pre-hybridization was done for 1-2 h and hybridization for ca. 16 h overnight at 68°C. Washing of the membranes was carried out for 1) 5 min at room temperature in 2 × SSC containing 0.5 % SDS, 2) for 30 min – 1 h in 0.1 × SSC plus 0.5 % SDS at 37°C, and 3) for 15 - 30 min at 68°C in 0.1 × SSC (20 × SSC: 3.0 M NaCl; 0.3 M Na_3 Citrate). After stripping off radiolabeled DNA, control hybridizations were performed (12) with a universal probe (13) at 40°C at least for 3 hours in 5 × SSPE (20 × SSPE: 3.6 M NaCl, 0.4 M Na_2HPO_4, 0.02 M EDTA pH 8.0) containing 5 × Denhardt (100 × Denhardt: 2 % w/v Ficoll, 2 % Polyvinylpyrrolidone, 2 % BSA) and 0.1% SDS. Membranes were washed two times for 5 min at room temperature and once for 5 min at 40°C in 6 × SSC with 0.5 % SDS.

2.7 Quantitation of Hybridization

The total intensities of digitalyzed hybridization signals were measured with the image analysis program SigmaScan Pro 5. The calculation was standardized by using DNAs extracted from a *Lactobacillus delbrueckii* subsp. *bulgaricus* Kt4 culture of known numbers of cfu (colony forming units) and defined optical density, 0.7 and/ or 1.0 OD_{620}, respectively, representing middle or end of exponential growth phase. The hybridization signal intensities obtained from three different amounts of that DNA were correlated with the respective number of cfu. The counts of the species *L. delbrueckii* in the chyme samples resulting from those calculations were expressed as "calculated cfu" per gram chyme (wet weight) or as percent proportion of total eubacteria DNA.

3. Results

From minipigs fed three different diets on three consecutive days four chyme samples were taken in rough hourly intervals starting three hours after intake of each diet. The experiment was repeated one week later. Three different amounts of total DNA extracted from each of the digesta samples were immobilized on the membrane. This was done for two reasons: first to control possible pipetting errors and second to expand the detection limits of the assay, as the proportion of the positively reacting DNA in the samples was unknown.

The first hybridization was carried out with a species-specific *L. delbrueckii* probe and the second one with an universal eubacteria probe after stripping off the membranes. Hybridization signals of the two animals are shown in Fig. 1 and 2. Measurement of total intensities of the hybridization signals was carried out in that way that only one of the three spots – and not the arithmetic middle of all spots – was used for the subsequent calculation. The total intensity of the respective spot chosen had to be in the expected relation to at least one of the other spots. Those spots that did not fit in a series of two others were not considered.

When feeding a semisynthetic diet to individual 1, calculated *L. delbrueckii* counts appeared to be near or slightly above the detection limits at a magnitude of 10^6/g digesta (Fig. 3). When yoghurt with inactivated starter cultures was applied, they obviously increased to a magnitude of more than 10^7 calculated cfu/g chyme 4-6 hours after ingestion. When feeding yoghurt with viable *L. delbrueckii,* calculated cfu increased reproducibly over 10^7/g chyme after 5 hours and reached nearly 10^8/ g chyme after 6 and 7 hours postprandially. Assessing the proportion of *L. delbrueckii* DNA compared to the total bacterial DNAs, *L. delbrueckii* constituted at most 3% on days with semisynthetic diet (highest values in week 1 and 2), but increased to 6% after 6 hours (highest value in week 1) and to 18% after 4 hours (highest value in week 2) when heat-treated yoghurt was fed. On day three, when applying yoghurt with viable bacteria, percentages of *L. delbrueckii* obtained were clearly above the values obtained during the days before. Highest values were 14% in week 1 and 29% in week 2.

In the second minipig (Fig. 2 and 4), the calculated cfu of *L. delbrueckii* dit not vary to that great extent when yoghurts in comparison to semisynthetic diets were fed, since the pigs obviously inhabited high numbers of endogenous *L. delbrueckii* at a magnitude of 10^7/g chyme even during application of semisynthetic diet. When heat-treated yoghurt or yoghurt with living starters was applied the calculated counts appeared to increase by a factor of two to three in comparison to the semisynthetic diet. The proportion of *L. delbrueckii* increased from about 16% and 10% for semisynthetic diet (highest values in week 1 and 2) to about 24% and 26% for heat-treated yoghurt, respectively. When yoghurt was fed on day 3, the proportion of *L. delbrueckii* was ca. 19% for both weeks. In the second week we observed only very weak binding of the eubacteria probe for samples from the semisynthetic meal. We do not have an explanation for this.

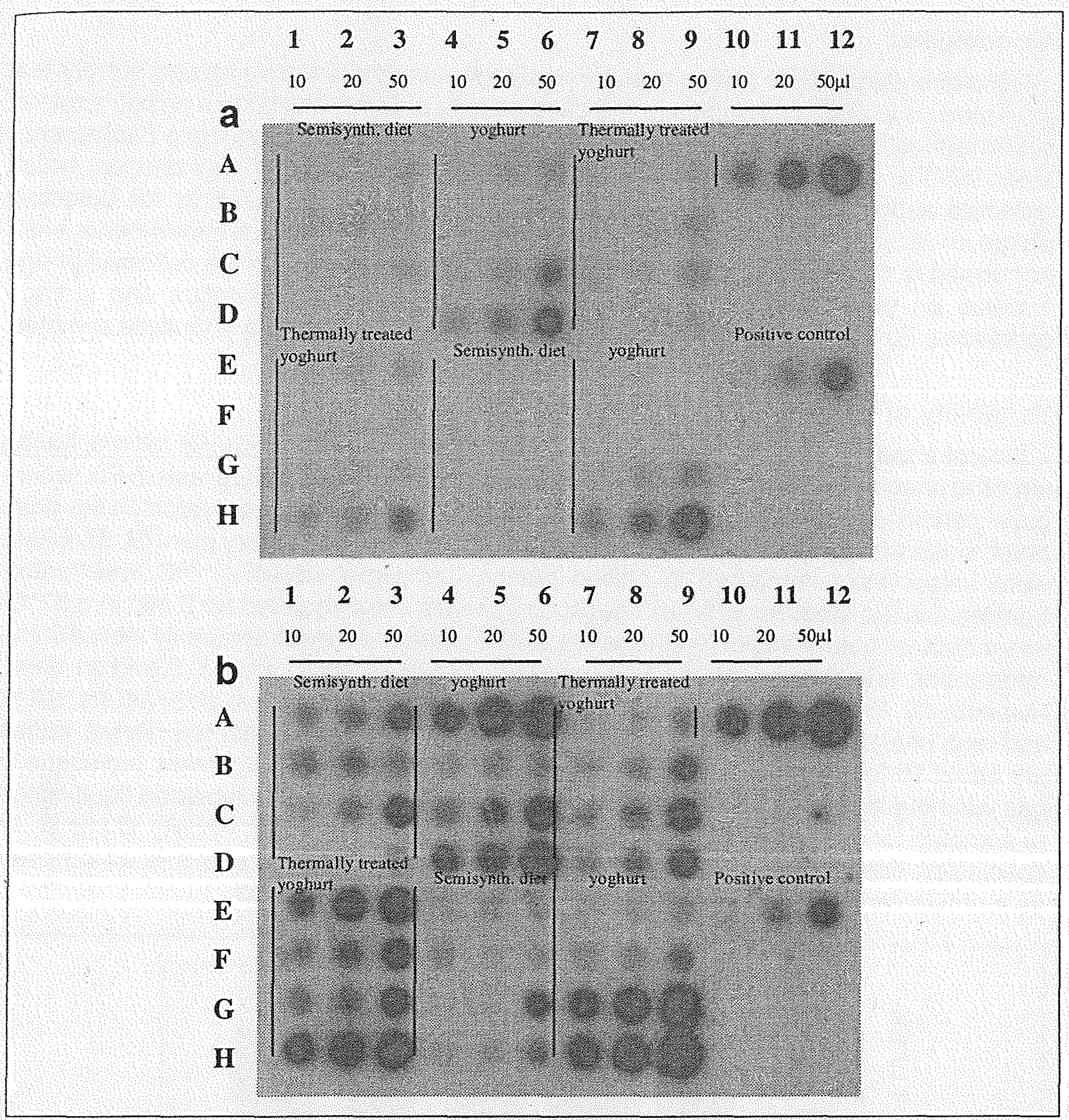

Fig. 1: Hybridization results obtained with DNA extracted from intestinal contents (terminal ileum) of fistulated Göttingen minipig 1 during several hours (four samples per day and diet with one exception) after feeding of a semisynthetic diet Sacas 15, thermally treated plain yoghurt and yoghurt containing living starter cultures. Feeding experiments were carried out on three subsequent days and repeated once one week later. In a) a hybridization probe species-specific for *Lactobacillus delbrueckii* was used. In b) an universal probe for eubacteria was used on the same membrane after stripping off the first probe. Lanes 1 - 3; 4 - 6, and 7 – 9: 10, 20, 50 µl of DNA solution prepared from each sample was spotted, respectively. A – D (1 – 3): DNA extracted from chyme after application of semisynthetic diet Sacas 15 (transition times between 3 h and 6 h). E – H (1 – 3): after application of thermally treated yoghurt (starters were inactivated, transition times between 3 h and 6 h). Lanes A – D (4 – 6): after application of yoghurt with living starter cultures (transition times between 3 h and 7.5 h). Repetition of the feedings: E – H (4 - 6): DNA extracted from chyme after application of semisynthetic diet Sacas 15 (transition times between 3 h and 5.5 h). A – D (7 - 9): after application of thermally treated yoghurt (starters were inactivated; transition times between 3 h and 6 h). E – H (7 - 9) and A (10 – 12): after application of yoghurt with living starter cultures (transition times between 3 h and 6 h).
E 10 – 12: Positive control was 10, 20 and 50 µl of DNA solution. DNA was extracted from *Lactobacillus delbrueckii* subsp. *bulgaricus* Kt4 collected at OD_{620} = 1.0 and redissolved in the same amount of TE-buffer as the cellsuspension initially harvested.

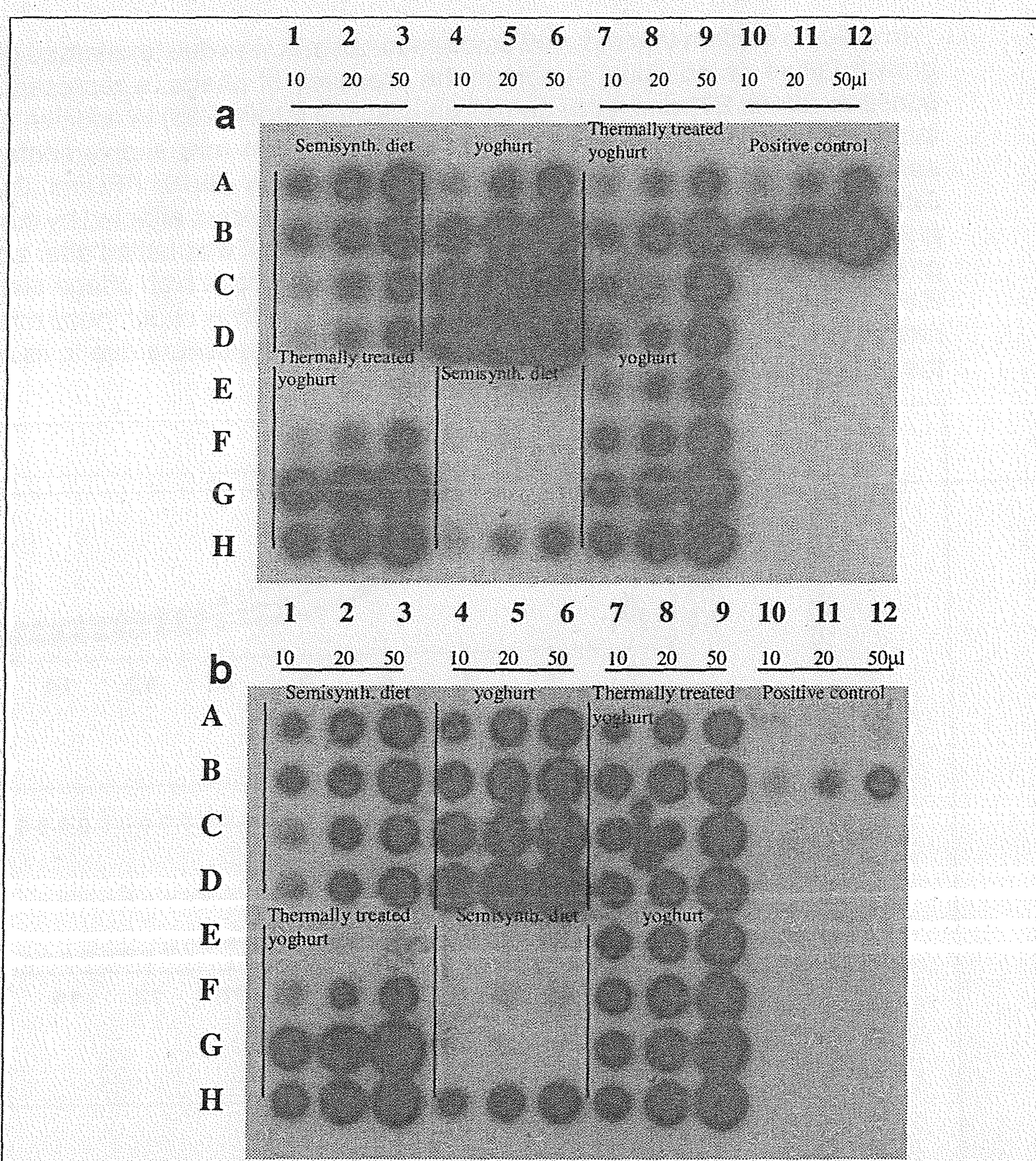

Fig. 2: Hybridization results obtained with DNA extracted from intestinal contents (terminal ileum) of fistulated Göttingen minipig 2 during several hours (four samples per day and diet) after feeding of a semisynthetic diet Sacas 15, thermally treated plain yoghurt and yoghurt containing living starter cultures. Feeding experiments were carried out on three subsequent days and repeated once one week later. In a) a hybridization probe species-specific for *Lactobacillus delbrueckii* was used. In b) an universal probe for eubacteria was used on the same membrane after stripping off the first probe. Lanes 1-3; 4-6, and 7–9: 10, 20, 50 µl of DNA solution prepared from each sample was spotted, respectively. A – D (1 – 3): DNA extracted from chyme after application of semisynthetic diet Sacas 15 (transition times between 3 h and 6 h). E – H (1 – 3): after application of thermally treated yoghurt (starters were inactivated, transition times between 3 h and 6 h). Lanes A – D (4 – 6): after application of yoghurt with living starter cultures (transition time between 3 h and 6 h). Repetition of the feedings: E – H (4 - 6): DNA extracted from chyme after application of semisynthetic diet Sacas 15 (transition times between 3 h and 5.5 h). A – D (7 - 9): after application of thermally treated yoghurt (starters were inactivated; transition times between 3 h and 6 h). E – H (7 - 9): after application of yoghurt with living starter cultures (transition time between 3 h and 7.5 h, respectively).

A, B 10 – 12: Positive control was 10, 20 and 50 µl of DNA solution. DNA was extracted from *Lactobacillus delbrueckii* subsp. *bulgaricus* Kt4 collected at OD_{620} = 0.7 (A) and 1.0 (B) and redissolved in the same amount of TE-buffer as the cellsuspension initially harvested.

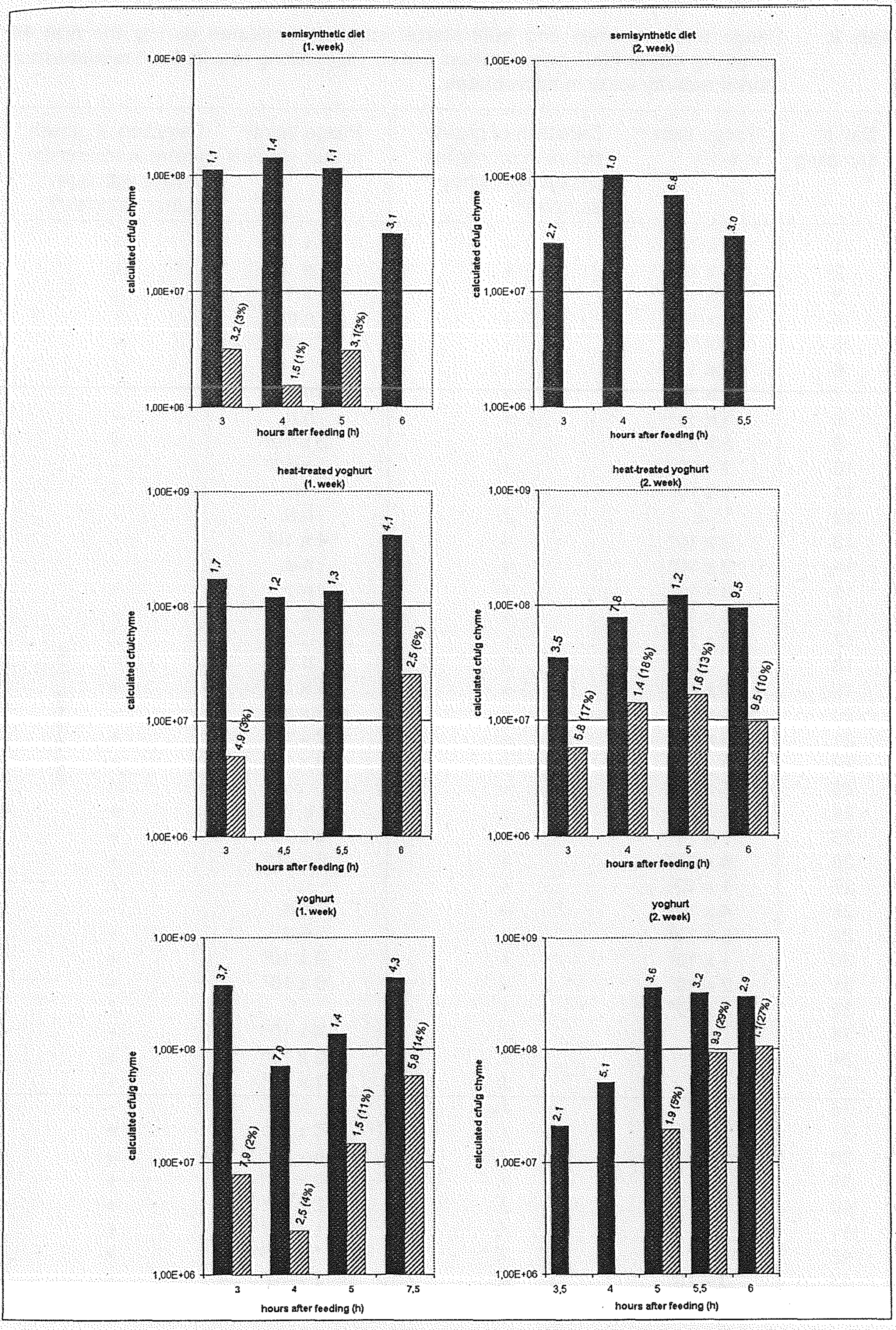

Fig. 3: Hybridization signals (presented in Fig. 1) obtained from miniature pig 1were analysed and quantified (see next page for further explanation).

Fig. 3: Different amounts of DNA (Fig. 1: Ia,b E10-12) extracted from *Lactobacillus delbrueckii* subsp. *bulgaricus* Kt4 (OD_{620}=1.0) of known bacterial counts were immobilized on the membrane. Depending on the probe specificity (universal probe for the domain bacteria or *L. delbrueckii* specific probe) signal strengths of these spots were used as basis for quantitation of eubacteria or *L. delbrueckii* DNA and subsequent calculation of eubacteria or *L. delbrueckii* counts in the unknown intestinal samples. ▩ These columns show the calculation of total counts of intestinal bacteria obtained from hybridization with an universal eubacteria probe. ▨ These columns show the calculation of *L. delbrueckii* obtained from hybridization with the species-specific probe. Three diets were applied on three subsequent days. Four samples were taken after different transition times. Experiments were repeated once in the following week.

Fig. 4: Different amounts of DNA extracted from *Lactobacillus delbrueckii* subsp. *bulgaricus* Kt4 (OD_{620} = 0.7 and 1.0, Fig. 2: IIa,b, A 10-12, B10-12, respectively) of known bacterial counts were immobilized on the membrane. Depending on the probe specificity (universal probe for the domain bacteria or *L. delbrueckii* specific probe) signal strengths of these spots were used as basis for quantitation of eubacteria or *L. delbrueckii* DNA and subsequent calculation of eubacteria or *L. delbrueckii* counts in the unknown intestinal samples (only the DNA extracted from the 0.1 OD_{620} was used for calculations). ▩ These columns show the calculation of total counts of intestinal bacteria obtained from hybridization with an universal eubacteria probe. ▨ Shows the calculation of *L. delbrueckii* obtained from hybridization with the species-specific probe. Three diets were applied on three subsequnt days. Four samples were taken after different transition times. Experiments were repeated once in the following week.

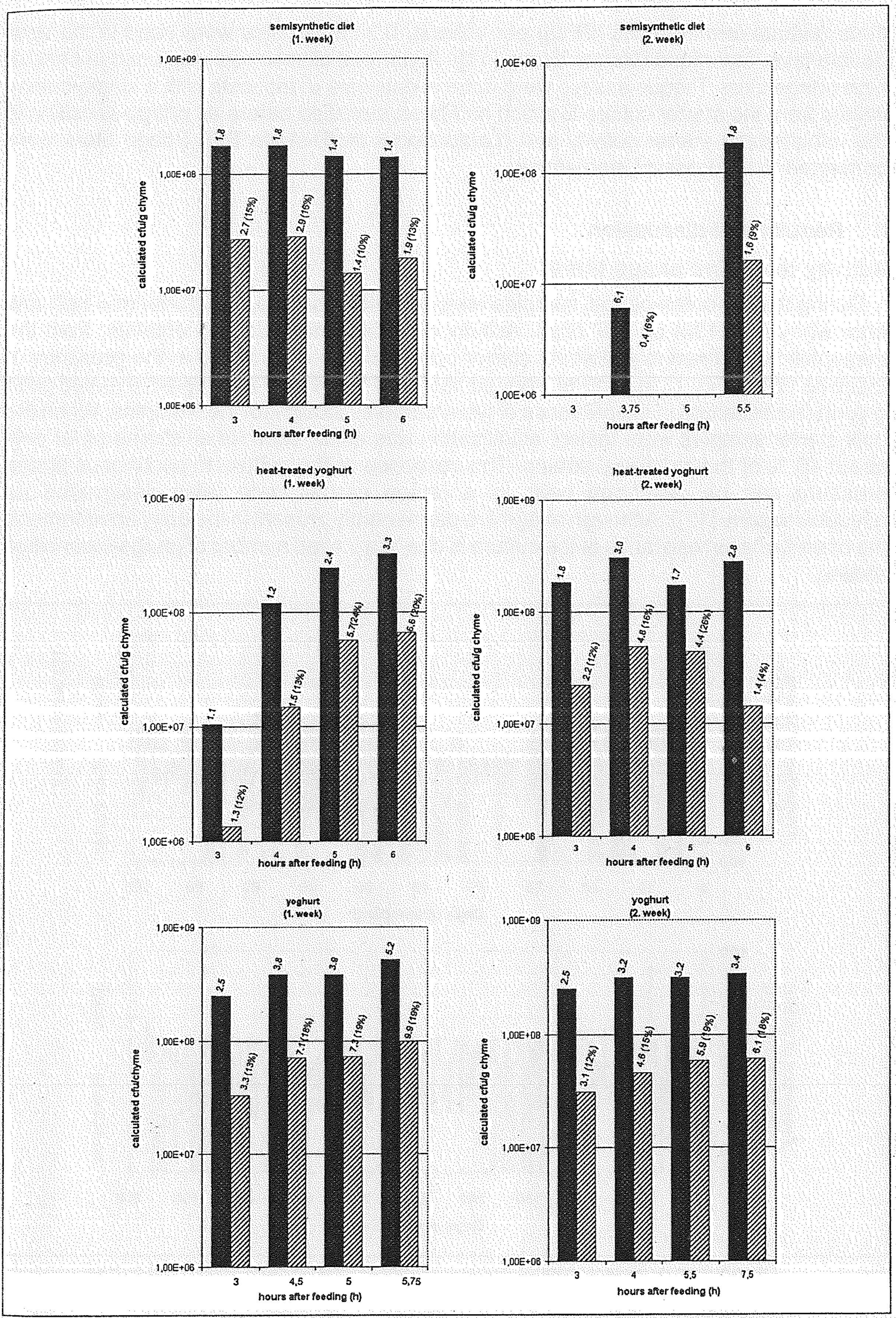

Fig. 4: Hybridization signals (presented in Fig. 2) obtained from miniature pig 2 were analysed and quantified (see preceding page for further explanation).

4. Discussion

Our intention was to observe variations in the *L. delbrueckii* population in the lower intestine depending on the diet applied and to describe influences – if there were any – of a yoghurt meal containing inactivated or viable starter cultures. For this purpose, hybridization experiments were performed with two hybridization probes and quantified with an image analysis system. Cfu obtained from plating experiments with pure cultures were correlated with the amount of DNA present in the culture at the time of plating, or - to be more precisely - with the total signal intensities obtained by the hybridization experiments. The *L. delbrueckii* hybridization probe used addresses only one single target per bacterial chromosome. Since the number of chromosomes varies in dependance of the growth phase of a culture (14, 15, 16) we chose two optical densities (0.7, 1.0 OD_{620}) reflecting the middle and the end of the logarythmic growth stage, respectively, as bases for calculation. There is neither knowledge about the growth stage a single bacterial species is in when it is part of the complex intestinal microbial population, nor how this stage develops during intestinal passage. Thus, it is a matter of consideration what is chosen as basis for the enumeration of bacteria in chyme samples. The calculations presented in this communication were correlated with the OD_{620} 1.0 *L. delbrueckii* subsp. *bulgaricus* culture. When OD_{620} 0.7 would have been the basis for calculation, the resulting cfu would have increased by about a factor of 2.

Moreover, the chain length of a microorganism can also vary with the growth phase (17, 18) and every single cell of a bacterial chain may provide more than one DNA target. Both effects increase the signal intensity of a hybridization in relation to the number of cfu, while a microbial chain is detected by plating on solid media as one cfu only.

A control hybridization using a universal probe for the domain bacteria was performed to assess the amount of eubacterial DNA. This probe was based on a 16S rDNA sequence (13). It is assumed that there are at least 500 bacterial species inhabiting the intestinal tract (3), the vast majority of which are unculturable and the copy number of rDNA operons from only a few of them is already known. Genome number, genome size and organization of *rrn* operons vary considerably among procaryotic species. Differences in genome size and in numbers of *rrn* operons have been detected for closely related species and even for strains of a single species (15, 19). The number of genes coding for 16S rRNA can vary from 1 to 15 (16), e.g. *L.delbrueckii* is known to possess four to five copies per genome (20). Consequently, the total eubacterial counts calculated on the basis of an hybridization with *L. delbrueckii* DNA using an universal probe must be regarded as provisional only. But we also tend to assume that the percent proportions of *L. delbrueckii* in the community, as presented in this communication were more likely underestimated.

The unexpectedly high proportion of *L. delbrueckii* cells detected in the chyme samples was not exclusively due to survival of the gastro-ileal passage of the starter bacterial taken in by the pigs. There is evidence that the lactose provided with the yoghurt stimulated endogenous lactic acid bacteria – here *L. delbrueckii* – for growth in the upper intestinal tract (Fig. 1 and 2, application of heat-treated yoghurt). This was analysed in more detail by applying PCR-systems that were able to identify not only the species *L. delbrueckii* but also both subspecies *lactis* and *bulgaricus* (4). *L. delbrueckii* DNA could be amplified after uptake of heat-treated yoghurt but not *L. delbrueckii* subsp. *bulgaricus*. The latter one was only found after feeding of yoghurt containing viable starters. This experiment in addition showed that the DNA of heat-inactivated starters did not act as target for hybridization and thus do not interfere with quantitation. Heat inactivated starters appear to be subject to rapid digestion in the intestine, as it was also found by other authors (21).

In addition to the variation of DNA target numbers, other factors exist which interfere with quantitation accuracy, as there are e.g. sample preparation, pipetting errors during application of the DNA onto a membrane, measurement of signal intensities of hybridization spots. The latter one alone showed variation of about ± 5-10% when the same spot was analysed several times. Since a detailed and comprehensive statistical analysis of all these factors was and is not possible, we are aware that quantitation results obtained by molecularbiological methods must be considered with advisible caution and criticism. However, it is our opinion that the relatively good reproducibility observed for the quantitation of different samples of one experiment can be regarded as confirmation of the (semi-)quantitative data.

Acknowledgement

S. Lick dedicates this publication to Bernd Hoffmann on the occasion of his retirement.

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6. Summary

Lick, S. , K. Drescher, K. J. Heller: **Quantitation by hybridzation of *Lactobacillus delbrueckii* in the terminal ileum of fistulated Göttingen miniature pigs after feeding of yoghurt.** Kieler Milchwirtschaftliche Forschungsberichte **53** (3) 193-206 (2001)

26 Quantitation by hybridization of *Lactobacillus delbrueckii* (yoghurt, intestine, identification and quantitation of a starter component).

Quantitation by dot blot hybridization of the starter culture component *Lactobacillus delbrueckii* in intestinal samples was carried out after applying a semisynthetic diet and two yoghurt meals either containing inactivated or living starters.

Feeding trials were carried out with two animals on three subsequent days and repeated one week later. The time course of the passage of the digesta was observed by taking four samples starting three hours postprandially. Hybridization probes were a *L. delbrueckii* specific probe and a universal probe for the domain bacteria. Enumeration of *L. delbrueckii* was expressed as "calculated counts" or as percent proportion of total eubacteria. Standardization was performed on the basis of the respective *L. delbrueckii* subsp. *bulgaricus* pure culture, that was grown to a defined optical density of known bacterial counts. The DNA extracted thereof was immobilized on each membrane and used as standard for the correlation of *L. delbrueckii* in the intestinal samples.

For one animal it was clearly shown that the counts deduced from hybridization intensity were highest after ingestion of yoghurt with living cultures. The increase obtained from yoghurt with inactivated starters was below that level. This concerned also the proportions of *L. delbrueckii* in relation to the total eubacteria (both weeks). As the *L. delbrueckii* probe could not distinguish between the subspecies *bulgaricus* ingested and the endogenous *lactis*, the increase is most probably caused by both subspecies in the latter case.

The second animal obviously inhabited a high background of endogenous *L. delbrueckii* strains that were able to ferment the semisynthetic diet. Nevertheless, a slight increase of calculated counts of *L. delbrueckii* concerning also its proportion among the whole flora was observed when one of the two yoghurts was ingested.

Problems inherent to molecular biological quantitation methods were discussed.

Zusammenfassung

Lick, S. K. Drescher, K. J. Heller: **Quantifizierung von *Lactobacillus delbrueckii* mit dot blot Hybridisierung im terminalen Ileum von Göttinger Miniaturschweinen nach Joghurtaufnahme.** Kieler Milchwirtschaftliche Forschungsberichte **53** (3)193-206 (2001)

26 Quantifizierung von *Lactobacillus delbrueckii* mit Dot blot Hybridisierung
(Joghurt, Darmtrakt, Identifizierung und Quantifizierung von Starterkulturkomponenten)

Der Anteil an *Lactobacillus delbrueckii* (Starterkulturkomponente) in Proben aus dem terminalen Ileum von Göttinger Miniaturschweinen wurde mit Hybridisierungssonden ermittelt. Die Hybridisierungssignale wurden mit Hilfe eines Bildanalysesystems (Sigma Scan Pro 5) ausgewertet. An drei aufeinanderfolgen Tagen wurde eine Semisynthetische Nahrung und Joghurt mit inaktivierten bzw. lebenden Starterkulturen verfüttert. Die Fütterungsversuche wurden mit zwei Tieren durchgeführt und in der darauffolgenden Woche wiederholt. Die Passage des intestinalen Inhaltes wurde zeitlich verfolgt. Vier Proben wurden in annähernd stündlichen Intervallen genommen, beginnend drei Stunden nach Aufnahme der Nahrung. Als Hybridisierungssonden dienten eine spezies-spezifische Sonde für *L. delbrueckii* und eine Sonde, die universell mit allen Eubakterien reagiert. Die Anzahl von *L. delbrueckii* wurde als „berechnete koloniebildende Einheiten" angegeben oder als Prozentanteil an der berechneten Gesamtmenge der Bakterien in der Probe. Eine Standardisierung der Berechnung wurde mit einer Reinkultur von *L. delbrueckii* subsp. *bulgaricus* Kt4 durchgeführt. Dieser Stamm war auch zur Fermentation des Joghurts eingesetzt worden. Während einer definierten Wachstumsphase wurden die Keimzahlen bestimmt und daraus DNA extrahiert. Diese DNA wurde zur Standardisierung auf jeder der Hybridisierungsmembranen aufgetragen. Die aus den Signalen ermittelten Schwärzungsintensitäten wurde mit denen des Probenmaterials verglichen und dann daraus die „Keimzahlen" berechnet.

Bei einem Tier konnte sehr deutlich gezeigt werden, daß die für *L. delbrueckii* ermittelten Zahlen am höchsten nach Aufnahme von Joghurt mit lebenden Kulturen waren. Nach Aufnahme von Joghurt mit inaktivierten Starterkulturen lagen sie niedriger, aber trotzdem deutlich erhöht gegenüber der semisynthetischen Diät. Dort lagen sie unterhalb oder nur knapp über der Nachweisgrenze. Dies bezog sich sowohl auf die berechnete Anzahl wie auch auf den berechneten Prozentanteil von *L. delbrueckii*. Da die Hyrididierungssonde speziesspezifisch reagiert und damit nicht zwischen der Subspezies *bulgaricus* (im Joghurt) und der im Darmtrakt bereits vorhandenen Subspezies *lactis* zu unterscheiden vermag, sind die beobachteten Anstiege im Probenmaterial auf beide Unterarten zurückzuführen.

Das zweite Tier besaß offensichtlich bereits einen hohen Hintergrund eigener *L. delbrueckii* Flora. Diese waren offensichtlich in der Lage, die semisynthetische Nahrung zu verwerten. Ein Anstieg des Anteiles von *L. delbrueckii* wurde dennoch beobachtet, wenn einer der beiden Joghurts gefüttert wurde.

Ungenauigkeiten bzw. systematische Fehler wurden erläutert, die bei molekularbiologischen Quantifizierungen auftreten.

Résumé

Lick, S. K. Drescher, K. J. Heller: **Quantitation par hybridation de *Lactobacillus delbrueckii* dans l'iléum terminal de porcs miniatures Göttingen après ingestion de yaourt.** Kieler Milchwirtschaftliche Forschungsberichte **53** (3) 193-206 (2001)

26 Quantitation par hybridation de *Lactobacillus delbrueckii* (yaourt, intestin identification et quantitation de la composante de culture de levain)

La quantitation par hybridation dot blot de la composante de la culture de levain *Lactobacillus delbrueckii* dans des échantillons intestinaux a été réalisée après un régime semisynthétique et l'ingestion de deux repas consistant de yaourt à ferments inactivés ou vivants.

Des essais d'alimentation ont été faits avec deux animaux pendant trois jours d'affilée et répétés une semaine plus tard. Le temps de passage de la quantité ingérée fut observé en prélevant quatre échantillons à des intervalles d'environ 1 heure, le premier trois heures après ingestion du repas. Comme sonde d'hybridation on a utilisé une sonde spécifique *L. delbrueckii* et une sonde universelle pour le domaine bactérien. La numération de *L. delbrueckii* était exprimée comme "calculated counts" (dénombrements calculés) ou comme pourcentage du total des eubactéries. La standardisation s'est faite sur la base de la pure culture de *L. delbrueckii* subsp. *bulgaricus* ayant obtenu une densité optique définie de dénombrement de colonies connues. L'ADN extrait était immobilisé sur chacune des membranes et utilisé comme valeur standard pour la corrélation de *L. delbrueckii* dans les échantillons intestinaux.

Pour un animal, il a été possible de clairement démontrer que le comptage obtenu pour *L. delbrueckii* était à son maximum après l'ingestion de yaourt à cultures vivantes. Le comptage après l'ingestion de yaourt à levain inactivé était en-dessous de ce maximum. Ceci était également valable pour les proportions de *L. delbrueckii* en relation avec le total des eubactéries (pour les deux semaines). Comme la sonde *L. delbrueckii* ne pouvait pas différencier entre la sous-espèce *bulgaricus* ingérée et le *lactis* endogène, il est fort probable que l'augmentation provient des deux sous-espèces dans ce cas.

Il saute aux yeux que le deuxième animal possédait déjà une grande quantité de *L. del-brueckii* endogènes capables de fermenter le régime semisynthétique. Cependant, une légère croissance de la proportion de *L. delbrueckii* était également constatée après l'administration d'un des deux yaourts.

Des problèmes inhérents aux méthodes de quantitation moléculaires-biologiques ont été discutés.