Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 30:138–152 (1991)

Ribonucleosides as minor milk constituents

E. Schlimme, K.-P. Raezke, and F.-G. Ott

Institute for Chemistry and Physics, Federal Dairy Research Centre, Kiel, FRG

Summary: Ribonucleosides are minor milk constituents and show a typical pattern which is assumed to be species-specific. As well as the unmodified components adenosine, cytidine, guanosine, inosine, and uridine, modified compounds such as Nl-methyladenosine and N6-carbamoylthreonyladenosine – products of the transfer RNA catabolism – have been identified and quantified in individual and bulk herd (race: German black pied) milk samples throughout a whole lactation period. The results of our longitudinal study have shown that – with the exception of the colostral phase – the levels of these minor constituents vary only slightly throughout lactation. These findings imply that ribonucleosides are useful for characterizing milk of different species and technological treatment. Ribonucleosides were determined and balanced, for example, in the course of the churning process, showing that the pattern of these minor milk constituents is useful as a "fingerprint" that allows differentiation between the three butter types defined in the German Federal Butter Ordinance.

Zusammenfassung: Ribonucleoside gehören zu den minoren Inhaltsstoffen der Milch und zeigen ein tierartentypisches Ribonucleosidmuster. Neben den unmodifizierten Komponenten Adenosin, Cytidin, Guanosin, Inosin und Uridin wurden modifizierte Verbindungen wie N1-Methyladenosin und N6-Carbamoylthreonyladenosin, die aus dem Transfer RNA-Katabolismus stammen, in Einzel- und Sammelmilchen einer kleinen Herde Deutscher Schwarzbunter nachgewiesen und quantitativ über eine gesamte Laktation bestimmt. Die Verlaufsstudie hat gezeigt, daß die Konzentrationsspiegel dieser minoren Komponenten mit Ausnahme der Kolostralphase über die gesamte Laktationsperiode nur einer geringen Schwankungsbreite unterliegen. Ribonucleosidmuster sind deshalb zur Kennzeichnung von Milchen verschiedener Herkunft und Verarbeitung geeignet. Beispielhaft wurden deshalb Ribonucleoside im Verlaufe des Butterungsprozesses bilanziert und gezeigt, daß diesen minoren Komponenten „finger-print"-Eigenschaften zukommen, die zur Differenzierung der von der Butterverordnung definierten drei Buttersorten geeignet sind.

Key words: Ribonucleosides, RNA catabolism, bovine milk, goat milk, colostral phase, lactation period, minor milk constituents, butter sera, intrinsic indicators, differentiation of butter types, HPLC

Schlüsselwörter: Ribonucleoside, RNA-Katabolismus, Kuhmilch, Ziegenmilch, Kolostralphase, Laktationsperiode, minore Milchinhaltsstoffe, Butterseren, intrinsische Indikatoren, Differenzierung von Buttersorten, HPLC

Introduction

Ribonucleosides are secreted as products of cellular RNA and ribonucleotide metabolism into physiological fluids such as blood, milk, and urine. During the last decade, common and modified ribonucleosides were characterized in a series of investigations as intrinsic compounds, especially in normal and pathological human urine on the basis of their concentration and pattern (2,5, 6,9–11, 13–15, 25, 27–29, 32, 33, 36, 37, 41). According to Schöch and coworkers (25, 26, 36, 37) urine concentrations, particularly of the modified and hypermodified ribonucleosides, are also suitable for measuring alterations of the metabolic status of the whole-body.

Unmodified and modified ribonucleosides have been detected as minor milk constituents (23, 24, 30, 40). In principle, ribonucleosides can enter milk by two pathways:

1) secretion as metabolic products from the lactating cell into the alveolar lumen, and
2) transfer as blood metabolites across the blood-milk barrier.

Though ribonucleosides are milk constituents, little is known about their importance from the lactobiochemical, nutritional, sensorial, and technofunctional points of view.

To provide the required information, we analyzed quantitatively on the basis of a longitudinal study (24) the concentration profiles of the individual ribonucleosides in raw bovine milk as a function of the lactation period (particulary of the colostral phase) under defined feeding conditions. In addition, we analyzed and balanced the ribonucleoside pattern in the course of the churning process (31) and compared ribonucleoside levels in butter sera (34) from different butter types.

Materials and methods

The apparatus consists of a modular, automated, high-performance liquid chromatography (HPLC) system combining chemoselective affinity and size exclusion properties in the pre-column with a reversed phase analytical column for quantifying the ribonucleosides in raw bovine milk. The two columns are connected via an automated six-port switching valve. The pre-column is filled with a modified phenylboronic acid substituted vinyl polymer (1) and has, under HPLC conditions, two functions:

1) the cis-diol groups of the ribonucleosides are bound chemoselectively and reversibly by forming, under slightly alkaline conditions, a cyclic diester, along with in conjunction with the phenyl boronate functional groups. The ribonucleosides are thus separated from the rest of the milk matrix.
2) The gel permeation properties of the pre-column material allow complete separation of the biological residual matrix, even if it contains proteins.

A complete on-line cycle for automated 24 h operation is characterized by a programmed sequence of five steps:

1) sample application via the autosampler. Chemoselective binding as well as enrichment of the ribonucleosides by the affinity ligand of the pre-column;
2) simultaneous, quantitative elution of the residual milk matrix constituents from the pre-column into the waste;

3) quantitative group-selective elution of the ribonucleosides from the pre-column by acidification of the immobilized cyclic boronate ester and simultaneous on-line transfer in a single, narrow elution band to the top of the series-connected analytical column;
4) separation of the ribonucleosides on the analytical column under reversed phase conditions;
5) reconditioning of the boronyl functionality for a new cycle during the analytical step.

The configuration of the automated dual column HPLC system, the gradient conditions in the analytical and wash program, as well as the sample preparation (milk and butter sera) has been described in detail elsewhere (2, 21, 22, 31, 32, 34). The validation of this HPLC system for the analysis of ribonucleosides occurring in raw milk was carried out in terms of the following criteria (20, 21, 22, 24): matrix-dependent recovery (97–100 %); repeatability (r) (7,8) for 20 determinations, and 95% statistical significance of matrix-dependent imprecision for lactophysiological ribonucleoside concentrations of up to 25 µmol/l (3–7 %); repeatability (r) of matrix-dependent imprecision for retention times (0.2–1.8 %); deviations from the true value (inaccuracy) in the range between 1–5 µmol/l (0.8–4.4 %) and between 5–84 µmol/l (0.5–8.8 %); detector response was linear in the range between 0.1–100 µmol/l (corresponding to 0.01–10 nmol ribonucleoside/run); determination limit/run (detection at 260 nm) was 1.5 pmol (uridine), 1.9 pmol (cytidine), 2.1 pmol

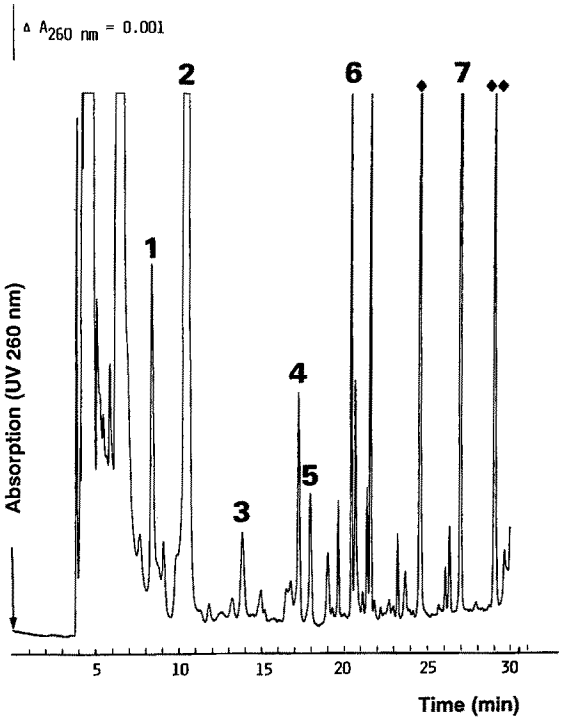


Fig. 1a. HPLC-diagram of the analysis of 100 µl of bovine milk (colostrum): 1 (cytidine), 2 (uridine), 3 (N1-methyladenosine), 4 (inosine), 5 (guanosine), 6 (adenosine), 7 (N6-carbamoylthreonyladenosine); ♦ m6Ado, ♦♦ m6,2Ado are externally added reference substances not originally occurring in bovine milk.

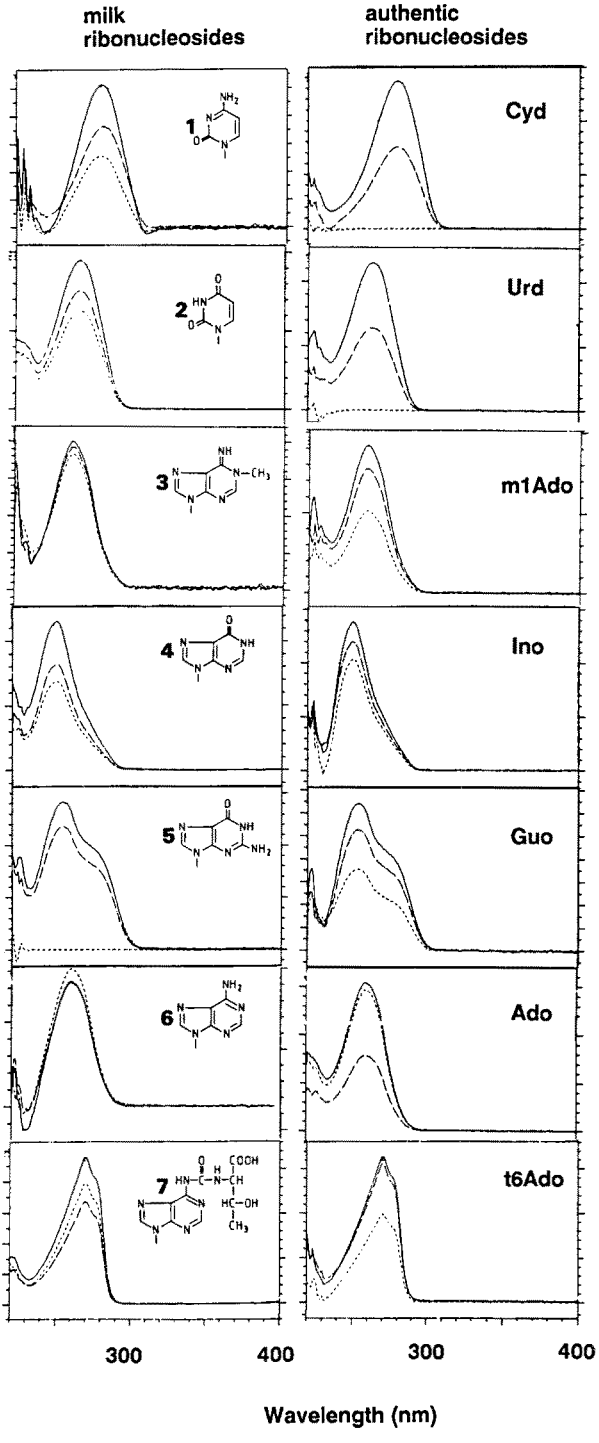


Fig. 1b. UV-spectra of the ribonucleosides (peak maximum (——), up-slope (....), down-slope (– – –): peaks 1–7 analyzed by HPLC of 100 µl of bovine colostral milk compared with authentic ribonucleosides showing isochromatographic behavior (compare "Materials and methods").

(N1-methyladenosine), 3.6 pmol (N6-carbamoylthreonyladenosine), 4.8 pmol (adenosine), 4.9 pmol (inosine), and 6.4 pmol (guanosine) in 100 µl sample volume.

For matrix dependent recovery the amount of ribonucleosides present in bovine milk was determined. The milk matrix was then spiked with defined amounts of ribonucleosides and analyzed again. To find out if all ribonucleosides are quantitatively preserved during preparation of the samples, which involves acidification of the raw milk (all individual milk samples immediately after milking; bulk herd milk samples not later than 1 h after milking), as well as of the butter sera (after melting of the butter and centrifugation) (31, 34) down to pH 3.5 for the purpose of sample preservation and subsequent deep freezing to −20 °C 2 ribonucleosides N6-methyladenosine (m6Ado) and N6-dimethyladenosine (m6,2Ado) not occuring in bovine milk were added to samples before acidification (Fig. 1). Recovery of these ribonucleosides with respect to sample preparation and HPLC analysis exceeded 93 %.

The authentic ribonucleosides were from Sigma Chemie (Munich). N6-carbamoylthreonyladenosine was synthesized according to (4) and characterized by elementary analysis, ^{1}H– and ^{13}C-NMR mass spectrometry and UV spectroscopy.

The study involved 8 German black pied cows which were fed on the basal ration (feed consists of silage made from winter barley, winter wheat, grass, and corn) ad libitum; concentrates were given according to the milk yields of the individual cows (23, 24). Measurements were started in mid-lactation and were continued until the next mid-lactation period. Combining both semiphases leads to a rather good overlap of the respective ribonucleoside levels (24), which allows the representation of the results in a whole lactation period. The ribonucleosides adenosine (Ado), cytidine (Cyd), guanosine (Guo), inosine (Ino), and uridine (Urd), as well as the modified components N1-methyladenosine (m1Ado) and N6-carbamoylthreonyladenosine (t6Ado) were quantitatively determined in the morning milkings of the cows on 3 days weekly (Monday, Wednesday, Friday) over a period of 446 days.

The peaks in the HPLC-chromatograms of the milk samples were examined before and after treatment of the milk with periodate. Such treatment oxidizes the ribonucleosides so that peaks attributable to this class of substance disappear from the chromatogram. Peaks were assigned to particular ribonucleosides on the basis of similarities of their chromatographic behavior and UV absorption spectra to those of pure substances (Fig. 1). The UV-spectra were measured with a programmable photodiode array detector (Model 994, Waters-Millipore, Eschborn). Characteristic peak shifting and quenching in HPLC analysis caused by enzymic (adenosine desaminase EC 3.5.4.4, nucleoside phosphorylase EC 2.4.2.1, Boehringer, Mannheim) and chemical modifications (Dimroth rearrangement of N1-methyladenosine to N6-methyladenosine) were also used for identifying the ribonucleosides.

Results and discussion

The studies have shown that, apart from the colostral phase, the ribonucleoside levels are constant throughout the whole lactation period, which is indicative of a typical ribonucleoside pattern in bovine milk. Results from analyses of other mammalian milks (20, 30) and measurements of nucleotide contents in milk from cows, sheep, and goats (12) suggest that this pattern is species-specific.

In addition to uridine, cytidine, and pseudouridine, which have already been determined in bovine milk (40), the following eight ribonucleosides have been identified in our group as minor constituents in bovine (24) and goat milk: adenosine, cytidine, guanosine, inosine, uridine, and the modified compounds N1-methyladenosine as well as N6-carbamoylthreonyl-

adenosine; very low concentrations (< 0.2 μmol/l) of N1-methylinosine were also found. The quantification was carried out on samples from single animals and samples from bulk milk.

Ribonucleosides in mature milk

The results of our longitudinal study involving a small herd of eight animals have shown that, with the exception of the colostral phase, the ribonucleoside levels are rather constant throughout a full lactation period, which is indicative of a typical ribonucleoside pattern in bovine milk. Table 1 summarizes the inter-individual mean values of the seven quantified ribonucleosides and, in addition, the minimum and maximum values measured throughout lactation, excluding the first 3 weeks post partum (24). The intra-individual ribonucleoside mean values for all the eight animals are reported elsewhere (24).

Figure 2 shows, for example, the inter-individual concentration profiles of some of the quantified ribonucleosides (24) cytidine, uridine, adenosine + inosine, and N6-carbamoylthreonyladenosine for a full lactation, including the colostral phase. The values for uridine and cytidine reported in (40) were found to be, respectively, five and two times higher than our findings. These anomalies may be explained by differences in the methods used. The method in (40) involves an elaborate and manually performed sample pretreatment (39), thus, postsecretory conversions are not to be excluded as discussed later on.

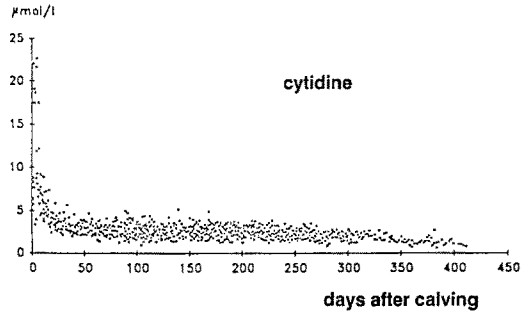
From the diagrams of all measured values for a given ribonucleoside it appears that the ribonucleoside level decreases during the colostral phase and, approximately three weeks post partum, reaches a level in milk which varies only slightly throughout lactation.

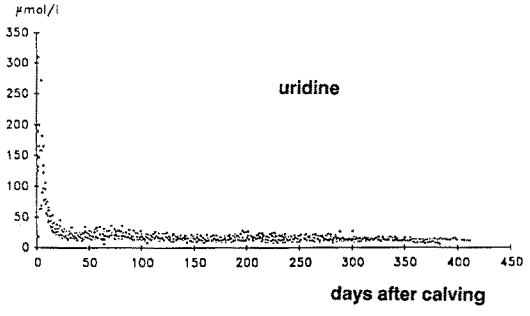
The inter-individual variation – in the following expressed as relative standard deviation in % (for standard deviations see Table 1) – is determined, above all, by the biological variation, apart from the percentage of

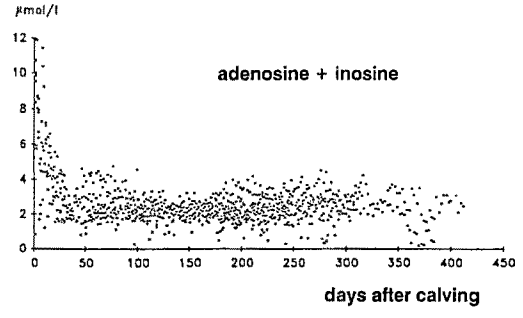
Table 1. Inter-individual values of ribonucleosides (μmol/l) in raw bovine milk.

	Ado	Cyd	Guo	Ino	Urd	m1Ado	t6Ado	Ado+Ino
c[a]	1.36	2.44	0.83	0.97	14.69	0.40	0.71	2.33
s[b]	0.67	0.78	0.32	0.42	4.73	0.08	0.14	0.74
s_r[c]	49	32	39	43	32	20	20	32
min[d]	0.06	0.53	0.10	0.01	3.63	0.17	0.40	0.13
max[d]	3.93	10.76	2.22	3.25	68.22	1.58	3.40	7.18

[a] Each mean value is the arithmetic mean of determinations (in duplicate) carried out on milk samples collected on 3 mornings per week (Monday, Wednesday and Friday) from each of eight cows (race: German black pied) throughout the whole lactation period, with the exception of the first 3 weeks post partum.
[b] Standard deviation in μmol/l.
[c] Relative standard deviation in % (including the percentage of imprecision of maximally 7 %; compare "Materials and methods").
[d] Minimum and maximum values measured throughout the whole lactation period with the exception of the first 3 weeks post partum.
(Cited with permission from (24).)







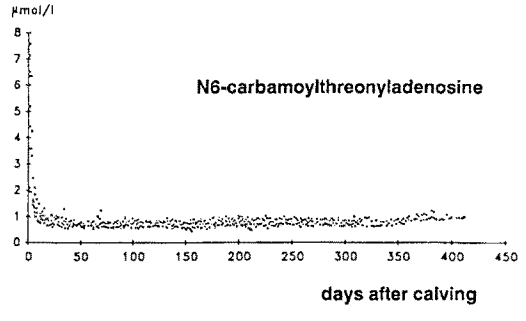


Fig. 2. Inter-individual values of ribonucleosides determined in duplicate 3 times per week throughout a whole lactation period in the morning milkings of eight cows (race: German black pied). Concentration profiles are shown for cytidine, uridine, adenosine + inosine and N6-carbamoylthreonyladenosine. (Reproduced with permission from (24).)

Table 2. Intra-individual mean values of ribonucleosides (μmol/l) in raw goat milk.

	Ado	Cyd	Guo	Ino	Urd	m1Ado	t6Ado	Ado+Ino
c[a]	3.44	n.d.	2.27	12.79	17.87	0.75	0.61	16.23
s[b]	0.60		0.50	3.56	5.35	0.45	0.07	3.83
min[c]	2.42		1.64	8.72	7.70	0.34	0.43	11.51
max[c]	5.17		3.93	25.13	29.70	1.90	0.71	28.85

[a]) Each mean value is the arithmetic mean of determinations (in duplicate) carried out on milk samples collected on 3 mornings per week (Monday, Wednesday and Friday) from a goat (race: German white "Edelziege") throughout 3 months in the middle of lactation

[b]) Standard deviation in μmol/l.

[c]) Minimum and maximum values measured throughout 3 lactation months.

imprecision of maximally 7 % as stated in the section "Materials and Methods". This is markedly lower for the two modified ribonucleosides than for the unmodified compounds. The relative standard deviation is approximately 20 % for m1Ado and t6Ado and about 35 % for Cyd, Guo and Urd. For Ado and Ino alone the relative standard deviation is 49 % and 43 %, respectively; for the sum of both ribonucleosides it ist markedly lower (32 %). This functional relationship is explained by the postsecretory activity of the milk enzyme adenosine desaminase. The variation coefficients for fat are around 20 %, for protein around 12 % and for lactose around 3.5 %. For the range of variation of the respective intra-individual ribonucleoside mean values of the eight animals see (24).

Table 2 compares the intra-individual mean values obtained for six ribonucleosides over a period of three lactation months in mature raw goat milk. From these findings and analyses of other mammalian milks (12, 20, 30) the ribonucleoside pattern is assumed to be species-specific.

Ribonucleosides in colostral milk

In the colostral phase the ribonucleoside contents in milk of all eight animals exceeded markedly (24) (Table 3) the mean content established for the lactation period except the first month post partum. This observation does not just apply for the colostrum during the first two days post

Table 3. Inter-individual values of ribonucleosides (μmol/l) in colostral milk.

	Ado	Cyd	Guo	Ino	Urd	m1Ado	t6Ado	Ado+Ino
c_7[a]	1.98	11.61	2.13	4.70	132.60	1.00	2.89	6.68
c_{14}[b]	2.61	6.02	1.96	2.94	58.22	0.53	1.06	5.55
c_{21}[c]	2.38	3.99	1.47	1.52	25.58	0.49	0.79	3.90

Each mean value is the arithmetic mean of determinations (in duplicate) carried out on milk collected on 3 mornings per week (Monday, Wednesday and Friday) from each of eight cows (race: German black pied) in the first (a), second (b) and third (c) lactation weeks post partum. (Cited with permission from (24).)

partum, but the ribonucleoside contents gradually decrease, reaching a level after approximately 3–4 weeks post partum which remains constant throughout lactation. In contrast to the unmodified ribonucleosides, the modified components m1Ado and t6Ado showed this pronounced dependence on lactation only in the first week of lactation. The rather high ribonucleoside concentrations (particularly, uridine) in colostral milk provokes questions about the lactobiochemical causes of this finding. It remains to be clarified, for example, whether the origin of milk ribonucleoside is humoral and/or local. In the first case, ribonucleosides penetrate from blood into milk via the blood-milk barrier (19, 42, 43); in the second case they result from metabolic processes in the mammary gland and are excreted from the lactating cell into the alveolar lumen or formed by postsecretory metabolic processes in milk. Thus, the high metabolic

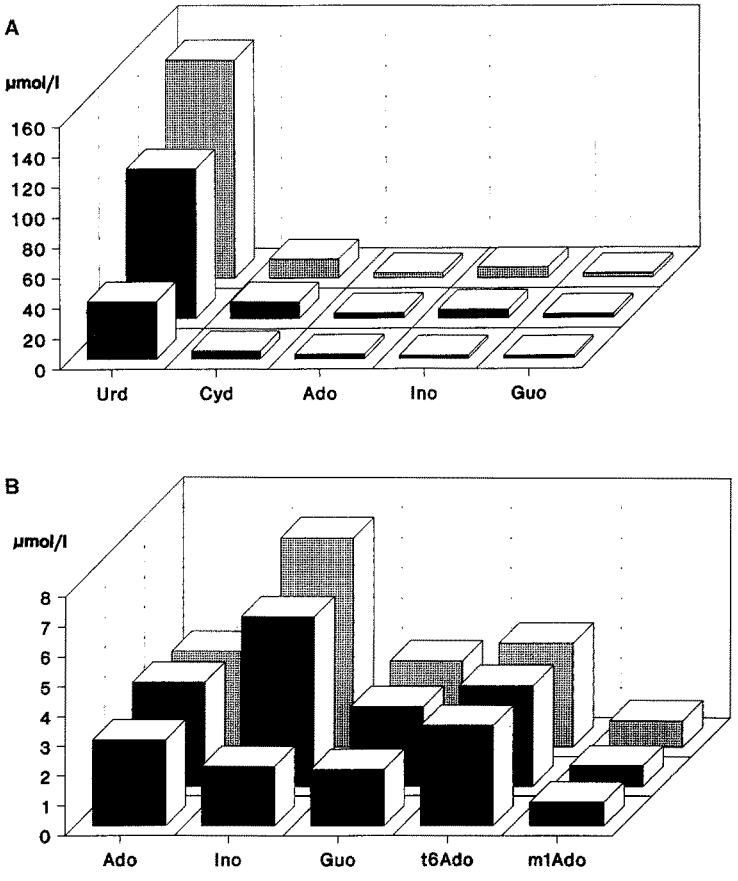


Fig. 3. Time-dependent alterations of the ribonucleoside concentrations due to postsecretory activities in colostral bovine milk: acidic preservation of the milk samples was carried out at 0 min (■), 30 min (▨), and 60 min (▤) after milking. A: unmodified ribonucleosides; B: modified ribonucleosides in comparison to unmodified purine ribonucleosides.

activity of the mammary gland should be considered along with the increased transfer rate of the ribonucleosides circulating in the blood through the blood-milk barrier in the first days post partum. During this period the colostrum contains a high proportion of blood constituents (19).

Table 3 summarizes the mean inter-individual ribonucleoside values obtained for the first three weeks of lactation. The initial ribonucleoside levels decrease markedly during the first month of lactation.

Determination of the ribonucleoside concentrations in 3-h intervals throughout the colostrum starting at the minimum of 1 h up to 48 h post partum indicates that some of the compounds such as uridine, adenosine and inosine oscillate slightly. In contrast to findings of Gil and Sanchez-Medina (12) concerning bovine milk nucleotides, no distinct maximum value was observed during the first two days after parturition. However, the concentration profiles of the seven ribonucleosides show, all in all, a decreasing tendency.

Postsecretory metabolic processes attributable to milk enzymes (e.g. alkaline phosphatase, adenosine desaminase), somatic cells, and microorganisms are markedly pronounced in colostral milk. Alterations of the ribonucleoside concentrations due to postsecretory activities are shown in Fig. 3. The ribonucleoside levels, especially of uridine and inosine, increase dependent on the time point of acidification (for the purpose of preservation) of the sample after milking.

Ribonucleosides in bulk herd milk

Table 4 summarizes the ribonucleoside concentrations determined in bulk milk of the small herd of eight cows throughout a period of 450 days. The bulk milk sample is made up of aliquots of the milk from each cow; corresponding to their appropriate milk yield including the colostrum. Since bulk herd milk samples were preserved by acidifying up to 1 h after

Table 4. Ribonucleoside values (μmol/l) in bulk bovine herd milk, including the colostrum.

	Ado	Cyd	Guo	Ino	Urd	m1Ado	t6Ado	Ado+Ino
c[a]	1.54	3.07	1.28	1.67	26.47	0.41	0.72	3.21
s[b]	1.37	0.83	1.91	1.72	29.40	0.05	0.10	2.76
s_r[c]	89	27	149	103	111	13	14	85
min[d]	0.16	1.62	0.18	0.21	9.53	0.26	0.47	0,50
max[d]	9.18	7.31	19.55	12.07	254.20	0.63	1.13	17.95

[a] Each mean value is the arithmetic mean of determinations (in duplicate) carried out on three collections per week (Monday, Wednesday and Friday) of bulk herd milk (from eight German black pied cows). The amount of milk taken from each cow for the bulk sample depended on the milk yield. Samples were taken throughout lactation (450 days) including the colostral-phase.

[b] Standard deviation in μmol/l.

[c] Relative standard deviation in % (including the percentage of imprecision of maximally 7 %; compare "Materials and methods").

[d] Minimum and maximum values measured in the bulk herd milk throughout the whole lactation (450 days) including the colostral phase.

milking, postsecretory ribonucleoside conversions must be taken into account.

As stated in the preceding section, postsecretory enzymic processes are pronounced in colostral milk and cause alterations in the ribonucleoside concentration and pattern (Fig. 3). Thus, this increased metabolic rate of ribonucleoside conversion readily explains the larger variation (expressed as relative standard deviation in %) in bulk herd milk samples containing portions of colostrum (Tab. 4) relative to those free of colostral milk (Tab. 5).

Comparing the variation of modified and unmodified ribonucleosides, it is noticeable that postsecretory conversion are mostly directed to unmodified ribonucleosides and not to the modified constituents such as m1Ado and t6Ado (Tables 4 and 5). Due to the colostrum portions in the bulk milk the compounds adenosine, inosine, uridine, and guanosine show an increased variation of up to 150 % (Table 4), whereas for the ribonucleosides in bulk milk without colostral milk (Table 5) the variation was in the same range found for the inter-individual ribonucleoside mean values for the eight animals (Table 1).

When the inter-individual ribonucleoside levels in raw mature milk are compared, it is striking that the contents of the pyrimidine ribonucleosides are approximately five times higher (about 17 µmol/l) than those of the purine ribonucleosides (about 3 µmol/l). This reflects, among other things, the lactobiochemical importance of uridine ribonucleotides, e.g. UDP-hexoses for lactose biosynthesis in the mammary gland. The findings agree with those reported in (12), in which was established rather high contents (up to 0.3 mmol/l) of UDP-glucose and UDP-galactose, mainly during the colostral phase, so that the tenfold increased uridine level (≈ 0.1 mmol/1, Table 3) during the first week of lactation is not surprising.

Table 5. Ribonucleoside values (µmol/l) in bulk bovine herd milk, excluding the colostrum.

	Ado	Cyd	Guo	Ino	Urd	m1Ado	t6Ado	Ado+Ino
c[a]	1.04	2.78	0.66	1.20	13.28	0.40	0.71	2.25
s[b]	0.52	0.58	0.18	0.28	2.43	0.06	0.08	0.60
s_r[c]	50	21	27	23	18	14	11	27
min[d]	0.16	1.62	0.31	0.51	9.53	0.27	0.56	0,79
max[d]	2.31	5.45	1.38	1.88	27.13	0.59	0.88	3.49

[a] Each mean value is the arithmetic mean of determinations (in duplicate) carried out on three collections per week (Monday, Wednesday and Friday) of bulk herd milk (from eight German black pied cows). The amount of milk taken from each cow for the bulk sample depended on the milk yield. Samples were taken throughout lactation (150 days) excluding the colostral-phase.

[b] Standard deviation in µmol/l.

[c] Relative standard deviation in % (including the percentage of imprecision of maximally 7 %; compare "Materials and methods").

[d] Minimum and maximum values measured in the bulk herd milk throughout the lactation (150 days) exluding the colostral phase.

Ribonucleosides as indicators in dairy product analysis

The experimental findings show that the pattern of ribonucleosides in mature milk varies only slightly throughout lactation. These minor constituents are, therefore, assumed to be intrinsic indicators, and might be useful as species-specific "finger prints" for quantifying adulterations of milk and milk products, and also for distinguishing different production processes.

The milk constituents adenosine, cytidine, guanosine, inosine, uridine, N1-methyladenosine, and N6-carbamoylthreonyladenosine were determined in raw milk, skim milk, butter milk, and butter serum, i.e., in the aqueous phases in the course of sweet cream and cultured butter processing (31). Table 6 summarizes the balancing of the ribonucleoside pattern in a typical course of butter making. The ribonucleoside concentrations found in butter sera were in the range of those determined in commercial butter samples (34). The average amount of ribonucleosides recovered in skim milk after cream separation was around 75 %.

Table 7 summarizes results obtained in comparative studies on the ribonucleoside pattern in melted butter sera of commercial samples (34) of the three different butter types defined in the German Federal Butter Ordinance (3).

A comparison of the ribonucleoside contents in the different butter sera obtained by melting the sweet and cultured cream butter shows that the ribonucleoside pattern permits differentiation between both butter types. A marked decrease in the ribonucleoside content of the cultured butter serum is clearly perceivable compared to that of sweet cream (Tables 6 and 7) which means that the ribonucleosides are rapidly metabolized during cultured cream butter making. Serum levels and pattern of ribonucleosides in softly acidified butter correspond to findings in sweet cream

Table 6. Balancing of the ribonucleoside content (µmol/l) in aqueous phases in the course of butter processing[a].

	Ado	Cyd	Guo	Ino	Urd	m1Ado	t6Ado
Raw milk[b]	0.43	7.52	0.95	1.13	56.12	0.48	0.85
Skim milk	0.26	6.73	0.59	0.57	50.74	0.45	0.76
Butter milk[c]							
– sweet cream	0.28	0.86	0.29	0.11	4.91	0.04	0.08
– cultured butter	0.01	0	0.01	0.03	0.97	0.01	0.08
Butter serum[d]							
– sweet cream	1.68	5.04	1.68	0.84	20.17	0.17	0.34
– cultured butter	0.73	0.15	0.36	0.07	3.65	0	0.36

[a] Results (determination in duplicate) of a typical course of sweet cream and cultured butter processing.

[b] Separation of bulk raw milk into skim milk and cream was carried out at 40 °C.

[c] Butter milk from pasteurized cream.

[d] Butter serum was obtained by melting the sweet cream and cultured cream butter at 50 °C followed by centrifugation (Gerber-centrifuge) and acidification (compare "Materials and methods").

Table 7. Ribonucleoside contents (μg/100 g butter)[a] in commercial butter samples.

	Ado	Cyd	Guo	Ino	Urd	Ado+Ino
Sweet cream						
(n = 5) $\bar{x}$	16.4	62.0	17.0	8.4	119.6	24.8
s	3.0	20.0	5.4	6.6.	24.3	6.9
min	12.7	40.5	10.5	3.3	92.1	17.8
max	20.5	81.8	21.8	19.1	147.2	35.7
Cultured butter						
(n = 5) $\bar{x}$	3.3	5.4	3.2	1.7	39.0	5.0
s	0.5	2.7	0.5	1.1	5.0	1.2
min	2.5	1.9	2.5	0.9	33.4	3.8
max	3.8	8.5	3.7	3.7	43.3	6.9
Softly acidified butter						
(n = 5) $\bar{x}$	8.2	26.7	7.9	3.2	60.5	11.4
s	7.6	18.2	8.7	1.6	35.4	9.1
min	2.0	3.0	1.0	1.0	8.2	3.0
max	19.6	44.5	22.3	5.2	98.5	24.8

[a] Each mean value is the arithmetic mean of ribonucleoside determinations (in duplicate) of five butter samples. The values given in μg ribonucleoside/100 g butter were derived from μmol ribonucleosides/l butter serum on the basis of the water content of the appropriate butter sample (35).

butter sera with exception of one sample which showed ribonucleoside as well as citric acid (35) concentrations typical for cultured butter serum (16, 17).

Though the number of commercial butter samples analyzed is rather limited, it may be concluded that a traditionally cultured butter is characterized by contents per 100 g butter of nucleosides of uridine < 50 μg, cytidine < 10 μg, adenosine + inosine < 10 μg, and guanosine < 5 μg.

Along with the established chemical indicators such as acetoin and diacetyl (18), as well as lactic- and citric acid (16, 17), ribonucleosides as intrinsic minor milk constituents can be used as chemical indicators, thus allowing a discriminating butter analysis (34, 35) and, hence, a differentiation between the butter types.

References

1. Boos KS, Wilmers B, Sauerbrey R, Schlimme E (1986) Deutsches Patent P 3 617 805.5
2. Boos KS, Wilmers B, Sauerbrey R, Schlimme E (1988) J Chromatogr 456:93–104
3. Butterverordnung vom 16. 12. 1988, Bundesgesetzblatt Teil 1, 2286–2295 (1988)
4. Chheda GB, Hong CI (1971) J. Med. Chem. 14:748–753
5. Chheda GB (1975) In: Fasman G (ed) Handbook of Biochemistry and Molecular Biology: Nucleic Acids CRC Press, Boca Raton, pp 251–270
6. Davis GE, Suits RD, Kuo KC, Gehrke CW, Waalkes TP, Borek E (1977) Clin. Chem. 23:1427–1435
7. DIN, ISO-Norm 5725 (1981)
8. Doerffel K (1987) Statistik in der Analytischen Chemie, VCH Verlag, Weinheim

9. Gehrke CW, Kuo KC, Davis GE, Suits RD, Waalkes TP, Borek E (1978) J Chromatogr 150:455–476
10. Gehrke CW, Zumwalt RW, Kuo KC (1984) Clinical Liquid Chromatography. In: Kabra PM (ed), Vol 2, CRC Press, Boca Raton, pp 139–154
11. Gehrke CW, Kuo KC (1989) J Chromatogr 471, 3–36
12. Gil A, Sanchez-Medina F (1981) J Dairy Res 48, 35–44
13. Hagemeier E, Kemper K, Boos KS, Schlimme E (1984) J Clin Chem Clin Biochem 22:175–184
14. Hagemeier E, Boos KS, Kemper K, Schlimme E (1987) In Krstulovic A (ed) Handbook of Chromatography, Nucleic Acid and Related Compounds, CRC Press, Boca Raton, pp 105–118
15. Hartwick RA, Krstulovic AM, Brown PR (1979) J Chromatogr 186:659–676
16. Kelnhofer F, Ziesel D, Klostermeyer H (1984) Deutsche Molkerei-Zeitung 48, 1704–1709
17. Kelnhofer F, Klostermeyer H (1984) Deutsche Milchwirtschaft 27, 1049–1051
18. Kiermeier F, Lechner E (1973) Milch und Milcherzeugnisse, Parey-Verlag, Berlin, Hamburg
19. Larson BL, Heary jr HL, Devery JE (1980) J Dairy Sci 63:665–671
20. Raezke KP (1988) Naturwissenschaftliche Dissertation, Universität Paderborn
21. Raezke KP, Wilmers B, Boos KS, Schlimme E (1988) Kieler Milchwirtsch Forsch Ber 40, 53–62
22. Raezke KP, Boos KS, Wilmers B, Schlimme E (1988) Milchwissenschaft 43, 224–229
23. Raezke KP, Frister H, Pabst K, Schlimme E (1988) Milchwissenschaft 43, 294–298
24. Raezke KP, Schlimme E (1990) Z Naturforsch 45c, 655–662
25. Sander G, Wieland J, Topp H, Heller-Schöch G, Erb N, Schöch G (1985) Clin Chim Acta 152, 355–361
26. Sander G, Hülsemann J, Topp H, Heller-Schöch G, Schöch G (1986) Ann Nutr Metab 30, 137–142
27. Savoia M, Russo T, Rippa E, Bucci L, Mazzeo F, Cimino F, Salvatore F (1986) J Tumor Marker Oncol 1, 61–68
28. Schlimme E, Boos KS, Weise M (1981) Clin Chem Clin Biochem 19, 55–60
29. Schlimme E, Boos KS, Hagemeier E, Kemper K, Meyer U, Hobler H, Schnelle T, Weise M (1986) J Chromatogr 378, 349–360
30. Schlimme E, Boos KS, Frister H, Papst K, Raezke KP, Wilmers B (1986) Milchwissenschaft 41, 757–762
31. Schlimme E, Raezke KP, Peters KH (1989) Kieler Milchwirtsch Forsch Ber 41, 243–251
32. Schlimme E, Boos KS (1990) Modified Nucleosides in Cancer and Normal Metabolism. In: Gehrke CW, Kuo KC (eds) Journal of Chromatography Library, Vol 45C, C115–C145, Elsevier Publ, Amsterdam
33. Schlimme E, Boos KS, Schwarzenau E, Frister H, Ott FG, Raezke KP, Wilmers B (1990) Nucleosides & Nucleotides 9, 407–410
34. Schlimme E, Schneehagen K, Ott FG (1990) Milchwissenschaft 45, 654–657
35. Schlimme E, Raezke KP, Ott FG, Schneehagen K (1990) DMZ-Lebensmittelindustrie und Milchwirtschaft 26, 856–860
36. Schöch G, Sander G, Topp H, Heller-Schöch G (1986) Monatsschr Kinderheilkd 134, 647
37. Schöch G, Sander G, Heller-Schöch G, Topp H, Telaar B (1988) In Haschke F (ed) Protein in der Säuglingsernährung, Ferd. Enke Verlag, Stuttgart, pp 26–39
38. Schwarzenau E, Schlimme E, Boos KS, Ott FG, Raezke KP, Wilmers B, Hilfrich J, Schneider J (1990) Tumor Diagn & Ther 11, 198–203
39. Tiemeyer W, Erbersdobler H, Giesecke D (1981) Z Lebensm Unters Forsch 173, 301–305

40. Tiemeyer W, Stohrer M, Giesecke D (1984) J Dairy Sci 67, 723–728
41. Uziel M, Smith LH, Taylor SA (1976) Clin Chem 22, 1451–1455
42. Ziv G, Sulman FG (1975) J Dairy Sci 58, 1637–1644
43. Ziv G, Heavner JE (1984) J Vet Pharmacol Therap 7, 55–59

Received February 20, 1991

Authors' address:

Prof. Dr. E. Schlimme, Institut für Chemie und Physik, Bundesanstalt für Milchforschung, Hermann-Weigmann-Straße, 2300 Kiel