KATION-ANIONVERSCHIL IN MELKVEE-RANTSOENEN ALS METHODE OM URINE pH EN AMMONIAKEMISSIE TE BEÏNVLOEDEN

[CATION ANION DIFFERENCE IN DAIRY COW RATIONS AS A MEASURE TO INFLUENCE URINE pH AND AMMONIA EMISSION]

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Inhoudsopgave

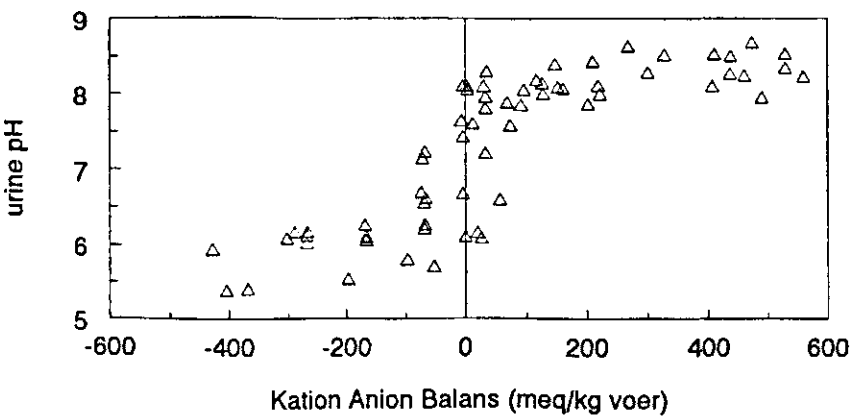
Samenvatting / Artikel gericht op praktijk

Een van de mogelijkheden om door middel van voeding de ammoniakemissie door melkvee te verminderen, is het verlagen van de zuurgraad, ofwel de **pH**, van de uitgescheiden urine. Uit fysische en chemische wetmatigheden kan afgeleid worden dat de pH van een lichaamsvloeistof in belangrijke mate bepaald wordt door het concentratieverschil in positieve kationen (Na, K, Ca, Mg) en negatieve anionen (Cl, S, P) in oplossing. Om deze reden geeft een sterk positieve of sterk negatieve kation anion balans (**KAB**) in het voer, een metabolische alkalose, resp. acidose, en als gevolg daarvan een basische, resp. zure urine.

Een analyse van uit de literatuur verzamelde gegevens leverde het op grond van de theorie verwachte S-vormige verband op tussen de pH van urine en de KAB in het voer. Zowel voor lacterende als niet-lacterende melkkoeien werd dit verband aangetoond, waaruit blijkt dat de invloed van de fysiologische status van het dier op de urine pH ondergeschikt is aan die van de KAB in het voer. In aanvulling hierop, is gepoogd het gevonden verband te verbeteren door de daadwerkelijke mineralenuitscheiding met urine te schatten op basis van afgeleide regressievergelijkingen of schattingen uit de literatuur. Dit gaf echter geen verduidelijking.

Zowel de KAB voor voersamenstelling als voor urine-uitscheiding werd op drie wijzen berekend: met Na, K en Cl, met Na, K, Cl en S, en met Na, K, Ca, Mg, Cl, S en P. De berekeningen met Na, K, Cl en S geven het best een verband weer tussen KAB en urine pH (figuur 1).

Figuur 1. Effect van kation anion balans in voer, berekend als (Na+K-Cl-2.S), op de pH van uitgescheiden urine pH



$$\text{Urine pH} = 5.72 + 2.57 / [\, 1 + e^{(-0.015 \times \text{DCAD})} \,] \qquad (R^2 = 0.69)$$

Een KAB op basis van alleen Na, K en Cl maakte het verband minder duidelijk, wat bevestigt dat S een belangrijk mineraal is in beschouwingen van de zuur-base balans. De zeer snelle omslag van een basisch naar een zure urine rondom pH 7 geeft aan dat voeraanpassingen of zouttoevoegingen een verandering in urine pH teweeg kunnen brengen van 2 eenheden bij een verlaging van de KAB van + 100 tot -100 meq/kg voer droge stof. Een verlaging van de pH van 8.5 (bij sterk positieve KAB, Figuur 1) naar 6.0 (bij -100 meq/kg voer droge stof, Figuur 1) geeft een verschuiving naar 250 maal meer ammonium dan ammoniak aanwezig in urine. Hoewel de pH snel stijgt in vers uitgescheiden urine op de stalvloer door urease-activiteit, kan aanpassing van de KAB in voer toch perspectieven bieden om de ammoniakconcentratie in urine te verlagen en dus de ammoniakemissie te verminderen. Hierbij dient wel opgemerkt te worden dat ruwvoer veel K bevat en ruwvoerrantsoenen voor melkkoeien doorgaans een sterk positieve KAB-waarde hebben. Bij dergelijke rantsoenen zou bij toevoeging van zouten de KAB nog steeds positief kunnen blijven en dus weinig effect hebben op de urine pH. Daarnaast dient nog onderzocht te worden of het langdurig voeren van een rantsoen met sterk verlaagde KAB geen gezondheidsproblemen met zich meebrengt. Hoewel hiervoor geen duidelijke aanwijzingen zijn gevonden zal experimenteel onderzoek dit eerst moeten uitwijzen.

1. Abstract

The effect was investigated of the dietary cation anion difference (DCAD) on the pH value of urine excreted by dairy cows. A sigmoidal relationship was established from experimental data of 23 feeding trials with 101 different treatments tested. The sigmoidal relationship can be explained from physical and chemical principles of an ionic solution. The physiological state of the cows (dry or lactating) had no effect on this relationship. A reduction in urine pH of two units appears possible with a reduction in DCAD from 100 to -100 meq/kg of feed. The DCAD values were calculated as (Na + K-Cl), (Na + K-Cl-2.S) and (Na + K + 2.Ca + 2.Mg-Cl-2.S-2.P). Calculation of (Na + K-Cl-2.S) explained observed values of urine pH best. In fitting a logistic equation and an equation based on theory, similar fitting results were obtained with 69.2% and 70.0% of observed variance explained, and a residual standard error of 0.61 and 0.60, respectively. The fitting results indicated a high sensitivity of urine pH for DCAD near the inflexion point at a DCAD of 0 meq/kg of feed and a urine pH of 7. Systematic differences between experiments caused a smoothening of the fitted curve, resulting in a less sensitive response of urine pH to changes in DCAD than actually observed within experiments. Relatively small changes in DCAD near the value of 0 meq/kg of feed DM had the largest effect on urine pH. Because a lower pH value decreases the ratio of ammonia/ammonium in urine there seem to be perspectives to lower the ammonia concentration in urine, and thereby the ammonia emission, by adapting DCAD. Beforehand, no serious implications on animal health are foreseen in the long term feeding of low DCAD diets. This still needs to be established by experimental research, however, because existing experimental evidence concerns the prevention of health problems of dairy cows prepartum.

2. Introduction

Several measures can be taken to reduce the emission of ammonia by dairy husbandry. Technical solutions to reduce ammonia emission already received much attention and are currently being practised in The Netherlands. These include covering manure storage pits, an altered animal housing and subsoil manure application (Monteny, 1996). However, nutritional measures may also be effective in further reducing ammonia emission (van Vuuren & Jongbloed, 1994). This has been confirmed in controlled experiments with dairy cows (Smits et al., 1995; van Vuuren et al., 1996). Nutritional effects on emission rates of ammonia are to be explained mainly from urine volume, urination frequency and urea concentration in urine (van der Aar et al., 1996).

An important factor to manipulate ammonia emission from dairy-cow houses is the ammonia concentration in the emitting fluid layer on the floor surface after the excretion of urine (Monteny, 1996). Chemical factors that determine the emission rate of ammonia are fluid pH which sets the ammonia/ammonium equilibrium, and the concentration of urea which is hydrolysed to ammonia by urease activity. Urea concentration may be estimated from the amount of urea excreted as estimated with protein evaluation systems and estimated urine volume (Bannink et al., 1998). However, a method to estimate urine pH accurately from available data on feed composition lacks.

For various animal species it has been established that urine pH is influenced by diet composition (Block, 1994; Kienzle, 1993; Mroz et al., 1996; Patience, 1990; Remer & Manz, 1993). Particularly the difference in cation and anion content in a diet is an important determinant of acid-base balance (Stewart, 1978 & 1983). Manipulation of the cation anion content of dairy cow diets is practised to prevent health problems peripartum (Block, 1984; Block, 1994; Goff & Horst, 1997, Van Mosel, 1991). In order to control urine pH by manipulating the cation anion content of a diet, the relationship needs to be precisely known. The aim of the present study was to investigate the relationship between feed composition and urine pH in dairy cows. In addition, some consideration was given to how a changed DCAD affects ammonia emission and animal metabolism and health.

3. Materials & Methods

A total of 23 feeding trials with 101 different rations were selected from literature (Bigner et al., 1996, 1997; Delaquis & Block, 1995a&b; Eickelberger et al., 1985; Escobosa et al., 1984; Goff & Horst, 1997; Joyce et al., 1997; Kilmer et al., 1981; McKinnon et al., 1990; Oetzel et al., 1991; Tucker & Hogue, 1990; Tucker et al., 1988a&b, 1991a&b; Van Mosel et al., 1993; Wang & Beede, 1990, 1992a&b; Waterman et al., 1991; West et al., 1991 & 1992) with observed values of feed intake, milk production, average number of days in milk, and urine pH (Table 1), dietary content of minerals (Table 2), and DCAD (Table 3). Experiments were performed on both lactating and nonlactating animals (pregnant as well as nonpregnant).

Table 1. Animal characteristics in experimental trials selected from literature.

	Average	Min	Max	n
Intake				
Dry matter (kg/d)	15.0 (5.2)	5.0	26.0	98
Milk				
Yield (kg/d)	15.5 (11.8)	0.0	39.0	101
Average days in milk (d relative to partus)	126 (85)	-24	242	70
Urine				
pH	7.4 (1.0)	5.4	8.7	101

Table 2. Dietary mineral content in experimental trials selected from literature.

Dietary content (g/kg of feed DM)	Average	Min	Max	n
Concentrate	497 (179)	0	1000	101
CP	158 (22)	88	219	97
Na	3.1 (2.8)	0.6	16.8	101
K	14.2 (5.9)	6.5	32.2	101
Ca	8.1 (2.6)	4.0	16.1	98
Mg	3.2 (1.2)	1.6	8.1	98
Cl	8.0 (3.9)	1.8	16.5	101
S	3.6 (1.9)	0.6	9.1	70
P	4.2 (1.1)	1.8	6.7	98

Table 3. Dietary Cation Anion Difference (DCAD) in experimental trials selected from literature.

DCAD (meq/kg feed DM)	Average (SD)	Min	Max	n
$(Na^+ + K^+ - Cl^-)$	266 (204)	-190	704	99
$(Na^+ + K^+ - Cl^- - S^{2-})$	50 (243)	-428	560	70
$(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + S^{2-} + P^{2-})$	562 (258)	-43	1210	64

It was assumed that mineral balances were zero (amounts of mineral apparently digested were completely excreted with urine or milk). Partitioning of the amounts of apparently digested mineral into excretion with milk and with urine was estimated with a set of empirical equations described previously by Bannink et al. (1998) for Na and K (Table 4). Additional linear regression equations for Cl were derived from results of van Vuuren et al. (1996) and De Jong (1996). Estimates for Ca, Mg, P or S were obtained from literature (NRLO, 1973; McDowell, 1992). Although it has been well established that the digestibility and the excretion rate of these minerals vary, constant values for the average situation were used in the present study (Table 4). Finally, 3.5% milk protein was assumed, containing 8 g Cysteine and 25 g Methionine/kg milk protein.

Dietary cation anion differences were related to observed urine pH, first, by calculating dietary cation anion difference (**DCAD**) from the analysed mineral content in the diet (meq/kg feed DM), and second, by calculating urinary cation anion difference (**UCAD**) from estimated amounts of minerals excreted with urine (eq/d). For every DCAD value a complementary UCAD was calculated. Amounts excreted with urine were estimated from mineral contents in the diet as described above. The valence of ions was taken into account and cation anion differences were calculated from Na, K, Mg, Ca, Cl, S and P contents in the diet (mmol/kg feed DM with DCAD calculation, mol excreted with urine/d with UCAD calculation). Cation anion differences were calculated either by (Na + K-Cl), (Na + K-Cl-2.S), or (Na + K + 2.Mg + 2.Ca-Cl-2.S-2.P).

Table 4. Regression equations for apparent digestibility, excretion with milk and excretion with urine of Na [1], K [1] and Cl [2].

Apparent Digestion [3]

D_{Na}	=	$-3.89 + 0.868 \times I_{Na}$	r.s.d. = 7.52 g/d	R^2 = 0.981
D_K	=	$-20.48 + 0.941 \times I_K$	r.s.d. = 14.7 g/d	R^2 = 0.989
D_{Cl}	=	$-21.90 + 0.956 \times I_{Cl}$	r.s.d. = 8.62 g/d	R^2 = 0.995

Estimates for Ca, Mg, P and S [4]

D_{Ca}	=	$0.5 \times I_K$
D_{Mg}	=	$0.2 \times I_{Mg}$
D_P	=	$0.6 \times I_P$
D_S	=	$0.75 \times I_S$

Milk Excretion [3]

M_{Na}	=	$3.68 + 0.250 \times$ Milk	r.s.d. = 1.39 g/d	R^2 = 0.437
M_K	=	$-7.03 + 1.903 \times$ Milk	r.s.d. = 2.32 g/d	R^2 = 0.943
M_{Cl}	=	$0.994 \times$ Milk	r.s.d. = 2.79 g/d	R^2 = 0.783

Estimates for Ca, Mg, P and S [4]

M_{Ca}	=	$2.4 \times$ Milk
M_{Mg}	=	$0.12 \times$ Milk
M_P	=	$1.5 \times$ Milk
M_S	=	$(0.025 \times 32.1/121.1 + 0.008 \times 32.1/149.1) \times 0.035 \times$ Milk

Urine Excretion [3]

U_{Na}	=	$3.29 + 0.925 \times (D_{Na} - M_{Na})$	r.s.d. = 10.7 g/d	R^2 = 0.958
U_K	=	$25.25 + 0.935 \times (D_K - M_K)$	r.s.d. = 31.4 g/d	R^2 = 0.948
U_{Cl}	=	$12.22 + 0.847 \times (D_{Cl} - M_{Cl})$	r.s.d. = 22.3 g/d	R^2 = 0.930

Estimates for Ca, Mg, P and S [4]

U_{Ca}	=	$D_{Ca} - M_{Ca}$
U_{Mg}	=	$D_{Mg} - M_{Mg}$
U_P	=	$D_P - M_P$
U_S	=	$D_S - M_S$

[1] Derived from Bannink et al., 1998.

[2] Estimated from data of De Jong (1996) and Van Vuuren et al. (1996).

[3] D = apparently digested (g/d); I = intake (g/d); M = excreted with milk (g/d); U = excreted with urine (g/d).

[4] Estimates made according to literature (NLRO, 1973; McDowell, 1992).

4. Results & Discussion

Relationships between DCAD or UCAD, and urine pH

A causal relationship between DCAD or estimated UCAD on the one hand, and acid-base balance and urine pH on the other hand, is to be expected according to physical and chemical principles (electro neutrality, dissociation constants, solubility of CO_2 gas). The theoretical considerations of a hypothetical ionic solution have been reviewed by Stewart (1978; 1983). The concentration of carbonate, bicarbonate, H^+ and OH^- are dependent variables in the system that are determined by the concentration difference in strong cations and anions, the concentration of dissolved CO_2 (or the partial gas pressure pCO_2) and the concentration of weak acid (total of dissociated and undissociated) as the independent variables. The value of every dependent variable is determined completely by the value of all three independent variables together. Neglecting the effects of dissolved CO_2 and weak acids, an induced excess of positively or negatively charged ions is expected to cause a major shift in solution pH (into alkaline and acidic, respectively) as demonstrated in Figure 1A. Because the solution is electro neutral, $[H^+]$ and $[OH^-]$ relate linearly to an increased difference in concentration of positively and negatively charged equivalents, except when $[H^+]$ and $[OH^-]$ approach 10^{-7} eq/L and the influence of the ion product of water becomes apparent (Figure 1B). The pH value of the solution with a water dissociation constant of 10^{-14} $(eq/L)^2$ is then defined by the following equation (Stewart, 1987; 1983):

$$[1] \qquad \text{Solution pH} = -\log\left[\sqrt{(10^{-14} + (\text{DCAD}/2)^2)} - \text{DCAD}/2\right]$$

As explained by Stewart (1978, 1983), changes in the concentration of weak acids are small in normal situations. Therefore, pCO_2 and the difference in strong cation and anion concentration in particular determine changes in pH of body fluids. Changes in pCO_2 are effectively and rapidly compensated by the respiratory system so that pCO_2 is normally maintained constant. This leaves the movement of strong ions to and from body fluids as the principal mechanism for acid-base interactions between body fluids. As stated by Stewart, it also becomes understandable then that the digestive tract and the kidney not only regulate mineral metabolism but also act as primary control sites for acid-base homeostasis.

The experimental data used in the present study clearly demonstrated a relationship between the DCAD analysed and observed urine pH. In Figure 2A, all available data on DCAD values were calculated by (Na + K-Cl-2.S). If CO_2 and acids or bases are assumed not to have influenced the effect of DCAD on pH at pH values near 7, the observed relationship between DCAD and urine pH corresponded to that expected from theoretical considerations (Stewart, 1978, 1983; Figure 1A vs. 2A). Mineral excretion with urine will not exactly reflect the diet contents because regulatory mechanisms operate on digestive and secretory processes in the gastrointestinal tract and on the urinary excretion of minerals. Nevertheless, by assuming that the DCAD of different diets caused a proportional concentration difference of cations and anions in urine fluid, the observed urine pH could be explained well from DCAD in the present study. Corresponding to theory, the largest change in pH per unit DCAD change occurred near a urine pH of 7 and a DCAD of 0 meq/kg of feed. These findings indicate that DCAD has the potential to evaluate dietary effects on acid-base balance and urine pH. Although an

considerable excess of cations or anions is excreted with urine (van Vuuren et al., 1996; De Jong, 1996) and thereby influences urine pH, the effect of dissolved CO_2 and acids or bases in urine can not be ruled out completely, but are probably important determinants for the asymptotic pH value that is reached with extreme DCAD values. Would data on all relevant components in urine (acids, anion, cations, CO_2) have been available, then urine pH could have been calculated more precisely according to physical and chemical principles described by Stewart (1978, 1983).

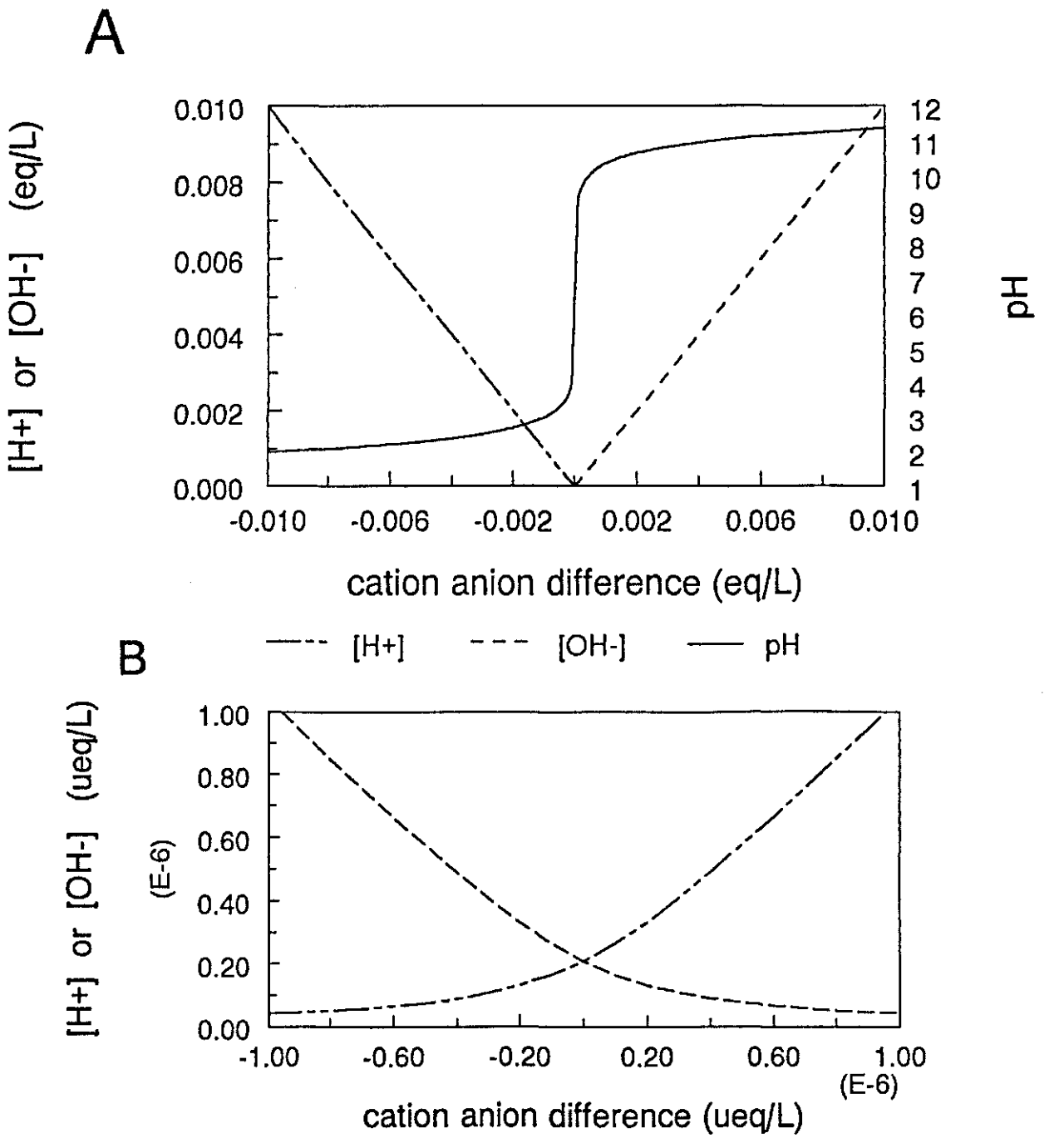


Figure 1. Theoretical impact of the difference in cation and anion concentrations on the pH of a solution (after Stewart, 1978, 1983).
A. Relationship for the whole range of urine pH, B. Relationship near pH 7.

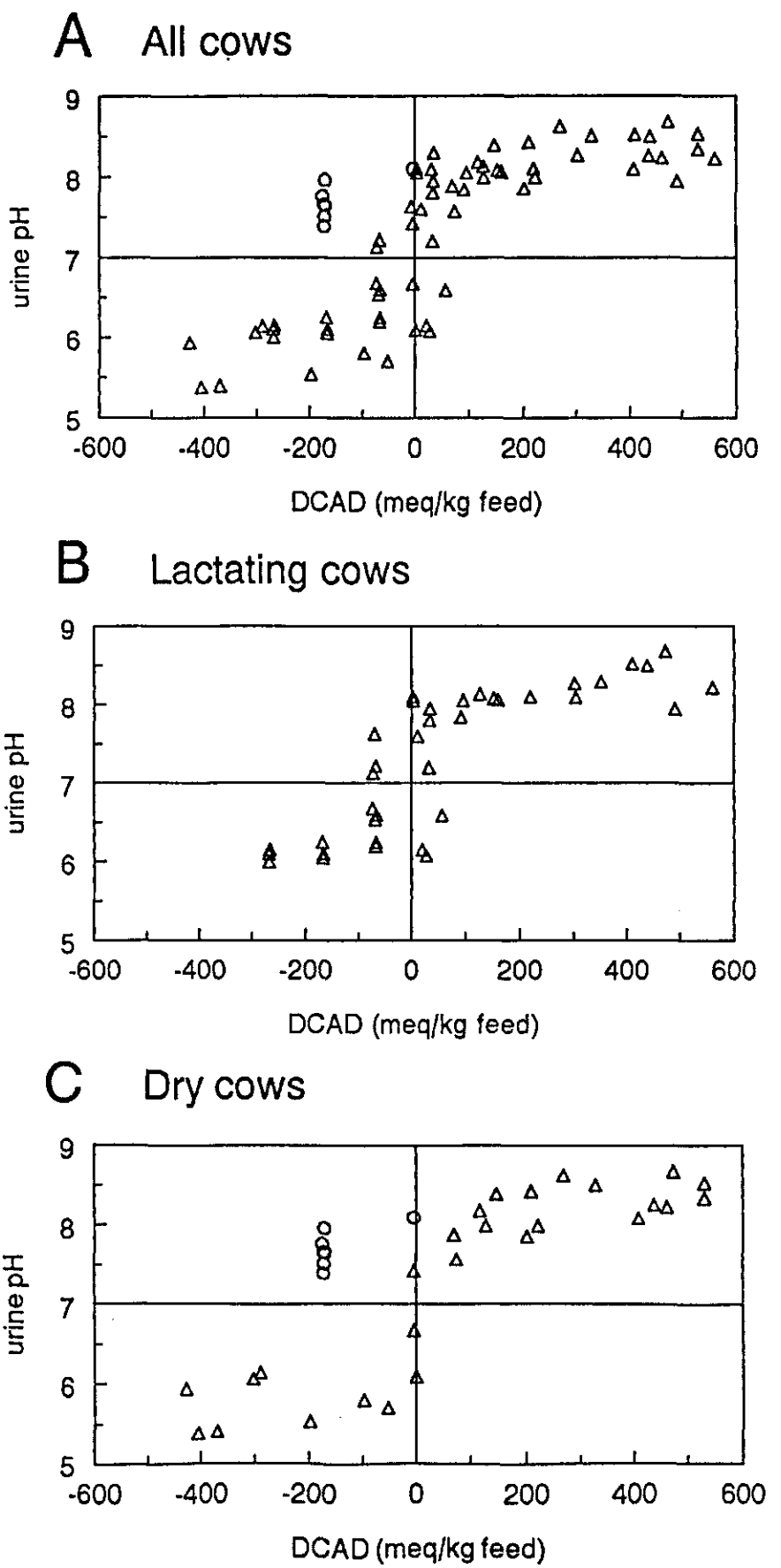


Figure 2. Published data (○, data from Oetzel et al., 1991; △, rest of data) on urine pH and dietary cation anion difference calculated (DCAD) as [Na + K-Cl-2.S]. A. All data, B. lactating cows, and C. dry cows.

An exception to the clear sigmoidal relationship were the data of Oetzel et al. (1991) with urine pH values almost 2 units higher than expected at these negative DCAD values. The obvious explanation for this deviation lies in the experimental procedure. In contrary to other studies, measurement weeks with one of six anion salts added to a control diet were separated from each other by only one week of control diet without salt addition. In studies on mineral metabolism, a period of one week is too short to prevent influences from previous treatments. This is nicely illustrated by the results obtained by Oetzel et al. (1991) in that only urine pH of the reference diet coincided with the sigmoidal relationship found, whereas all diets with salt additions deviated strongly in the direction of the reference diet because steady-state conditions were not yet reached (circles in Figure 2C).

Excluding the data of Oetzel et al. (1991), an empirical logistic equation was fitted to data of DCAD and urine pH (GENSTAT, FITNONLINEAR):

[2] $$\text{Urine pH} = p1 + p2 / [\,1 + e^{(-p3 \times (DCAD - p4))}\,]$$

(R^2 = 0.767; rse = 0.491; RSS = 14.49; df = 61)
(p1 = 5.808 ± 0.185; p2 = 2.525 ± 0.243; p3 = 0.0147 ± 0.0038; p4 = -12.5 ± 17.9)

with R^2 = proportion of variance accounted for by the fit, rse = residual standard error, df = residual degrees of freedom.

Investigation of the residuals revealed they were not uniformly distributed. At DCAD values where the asymptotic values of urine pH were reached, residuals were smallest whereas at DCAD values between -100 and +100 meq/kg of feed the residuals were largest. Weighing data by $1/DCAD^2 + 1$ gave a more uniform distribution of residuals. Although a uniform distribution of residuals is required for reliable parameter estimates, estimates remained almost the same:

[3] $$\text{Urine pH} = p1 + p2 / [\,1 + e^{(-p3 \times (DCAD - p4))}\,]$$

(R^2 = 0.696; rse = 0.608; RSS = 22.16; df = 60)
(p1 = 5.932 ± 0.189; p2 = 2.420 ± 0.263; p3 = 0.0177 ± 0.0055; p4 = 24.5 ± 16.7)

The large standard deviation of parameter p4 in equation 3 indicates that the estimate of this parameter was not significantly different from 0.0. Omitting this parameter in eq.4 resulted in comparable R^2 and RSS.

[4] $$\text{Urine pH} = p1 + p2 / [\,1 + e^{(-p3 \times DCAD)}\,]$$

(R^2 = 0.692; rse = 0.612; RSS = 22.85; df = 61)
(p1 = 5.722 ± 0.173; p2 = 2.573 ± 0.290; p3 = 0.0145 ± 0.0044)

Besides the empirical logistic equation, also the more mechanistic description of eq.1, based on chemical and physical principles, can be used in fitting the experimental data. Two parameters, p5 and p6, were introduced in eq.1, whose meaning must be something like the specific value of the ion product of water in urine and the factor by which DCAD becomes apparent in urine, respectively. Similar percentages of variance were explained by this theoretical relationship as compared to the fitting results of the logistic equation:

[5] $$\text{Urine pH} = -\log\left[\sqrt{(p6 \times 10^{-14} + (\text{DCAD}/(2 \times 10^{p5}))^2} - \text{DCAD}/(2 \times 10^{p5})\right]$$

($R^2 = 0.695$; rse = 0.609; RSS = 23.00; df = 62)
(p5 = 8.250 ± 0.126; p6 = 1.030 ± 0.346)

Because the estimate of p6 appeared not to be significantly different from 1.0 it was omitted in eq.6 and even improved the fit:

[6] $$\text{Urine pH} = -\log\left[\sqrt{(10^{-14} + (\text{DCAD}/(2 \times 10^{p5}))^2} - \text{DCAD}/(2 \times 10^{p5})\right]$$ (eq.6)

($R^2 = 0.700$; rse = 0.604; RSS = 23.01; df = 63)
(p5 = 8.258 ± 0.087)

In Figures 3A&B it is shown that both types of equations (eq. 4&6) describe a symmetrical sigmoidal relationship with the inflexion point at DCAD of 0 meq/kg of feed and urine pH of 7.0. The estimated upper and lower limit of urine pH with strong positive and negative DCAD values, appeared to be 8.3 and 5.7, respectively, with the logistic equation (eq.4). With the equation based on theory (eq.6), predicted urine pH continually increases and decreases with increasing and decreasing DCAD values. This seems to contradict with the experimental data as shown by the systematic deviation between the fitted and observed urine pH at high DCAD values (Figure 3B). Additional variables like CO2 and weak acids (Stewart, 1987 & 1983) must be taken into account to give an improved mechanistic explanation of the urine pH observed. Because of its potential to fit asymptotic pH values, the fitted logistic equation seems to be preferred at this moment to predict urine pH from DCAD in practical situations.

Also other factors, besides DCAD, possibly influence the regulatory mechanisms that ensure acid-base homeostasis, like for example the physiological state of dairy cows. However, a comparison of data obtained from lactating cows with those from dry cows did not reveal a difference in the relationship between urine pH and DCAD (Figure 2B vs. 2C). It seems the physiological state is a less important factor than DCAD in determining urine pH. However, differences in physiological state might cause a small shift in urine pH at small DCAD values near the inflexion point. In order to predict the effect of DCAD on urine pH accurately within the range of -100 to 100 meq/kg of feed, a precise positioning of the inflexion point is required and hence small effects of physiological state or other conditions on urine pH may become important. An indication of such a shift in inflexion point between experiments was in the

present study and is shown in Figure 3C. There seemed to be systematic differences between experiments. In order to test whether such differences between experiments were statistically significant, a separate logistic equation was fitted for every individual experiment within a single regression run. For this purpose, only experiments can be used that deliver enough data (more than 4 treatments) and have a wide enough range in DCAD values tested. Three experiments fulfilled these criteria (Tucker et al., 1988b & 1991b; Goff & Horst, 1997). Because residuals were uniformly distributed over DCAD values, weighing of data was not necessary. Finally, in order to let the iterations in the nonlinear regression routine converge to an appropriate fit, the parameters p2 (pH difference between lower and upper asymptotic pH value) and p3 (rate of increase of urine pH with increasing DCAD) were restricted to an identical value for all three experiments. From the fitting results obtained, the difference between these experiments appeared to be significant. The common p2 and p3 parameters were estimated to be 2.439 ± 0.183 and 0.0333 ± 0.0081, whereas for data from Tucker et al. (1988b), Tucker et al. (1991b) and Goff & Horst (1997) parameter p1 was estimated as 6.136 ± 0.080, 5.681 ± 0.199 and 5.752 ± 0.143, and parameter p4 as 17.5 ± 10.3, -69.41 ± 9.35 and 148.0 ± 25.2, respectively (R^2 = 0.955; RSS = 0.8540; rse = 0.202; df = 21). It is concluded that the estimates of p1 and p4 differed between these experiments. Moreover, the fitted response of urine pH to DCAD was about twice as large near the inflexion point than fitted for the whole data set (p2 estimate of 0.0333 vs. 0.0177). It appears that differences between experiments in the position of the inflexion point and in asymptotic pH values reached with extreme DCAD values caused considerable variation in the descending part of the sigmoidal curve. This variation could not be explained by a single sigmoidal curve fitted to all data simultaneously. It is not understood what experimental conditions or differences in methodology caused this variation between experiments.

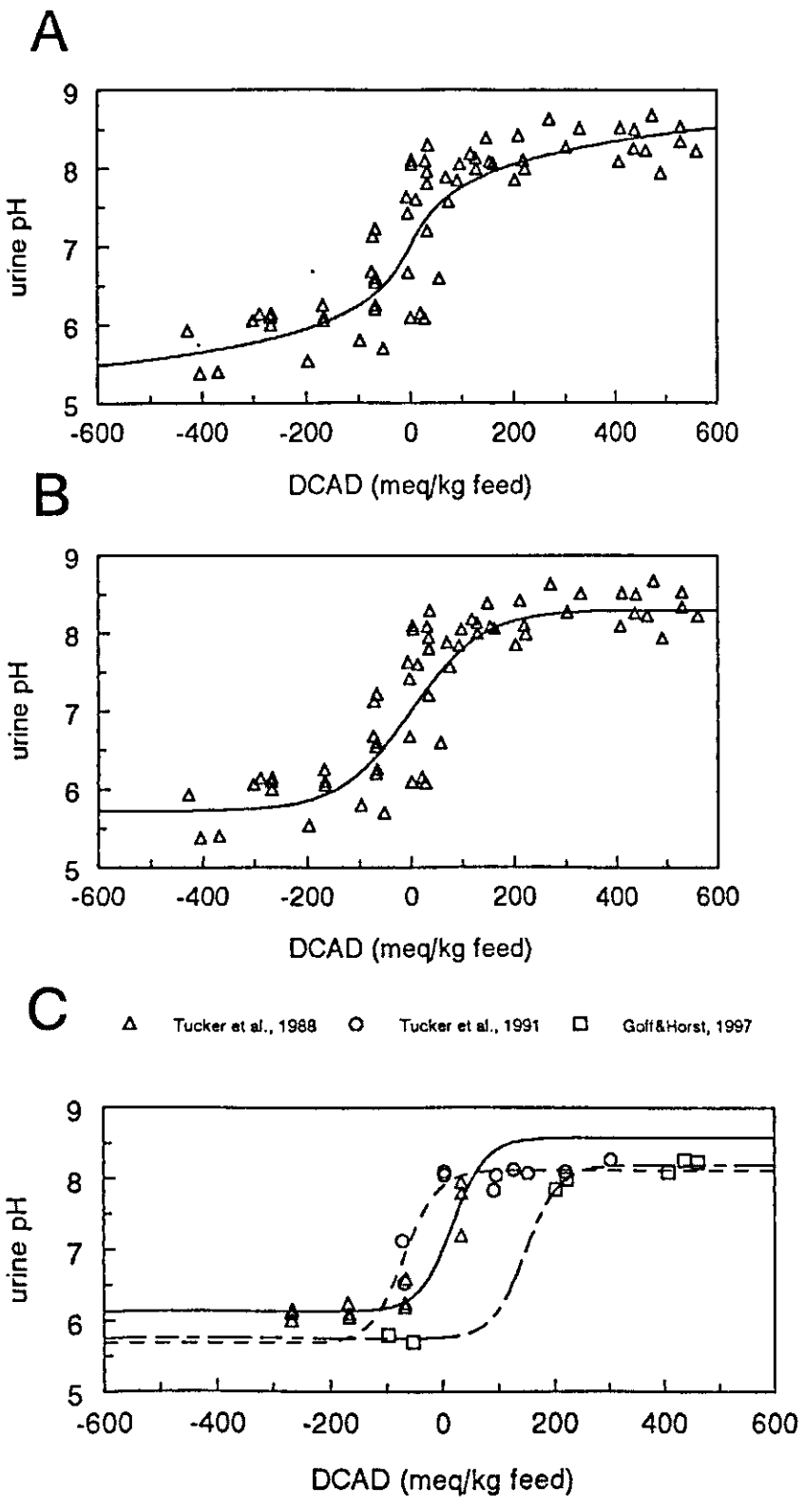


Figure 3. Relationship between dietary cation anion balance (DCAD) and urine pH.

A. Fit of logistic equation:

$$\text{urine pH} = 5.932 + 2.420 / [1 + e^{[-0.0145 * DCAD]}].$$

B. Fit of theoretical equation:

$$\text{urine pH} = -\log [\sqrt{(10^{-14} + (DCAD/(2 \times 10^{8.26}))^2)} - DCAD/(2 \times 10^{8.26})].$$

C. Simultaneous fit of separate logistic equations to three individual experiments of Tucker et al. (1988b & 1991b) and Goff & Horst (1997):

$$\text{urine pH} = 6.136 + 2.439 / [1 + e^{[-0.033 * (DCAD - 17.5)]}].$$

$$\text{urine pH} = 5.681 + 2.439 / [1 + e^{[-0.033 * (DCAD + 69.4)]}].$$

$$\text{urine pH} = 5.752 + 2.439 / [1 + e^{[-0.033 * (DCAD - 148.0)]}], \text{ respectively.}$$

Alternatives of DCAD

In the results discussed so far, DCAD was calculated by (Na + K-Cl-2.S). In evaluating the relationship between observed urine pH and three methods of DCAD calculation in Figure 4, the most evident relationship was obtained by this way of calculating DCAD (Figure 4B). Omission of S clearly distorted the relationship (Figure 4A). Although Mg, Ca and P in theory also contribute to cation anion difference, the relationship completely vanished with their inclusion (Figure 4C). This distortion of the relationship can be explained by a far less complete and more variable digestibility of Mg, Ca and P (McDowell, 1992) compared to that of Na, K, and Cl, and to a lesser extent to that of S (Table 4). As a consequence, the dietary contents of Mg, Ca and P contributed less and probably in a more variable manner to a cation anion difference affecting urine pH. It can be concluded that the inclusion of dietary contents of Mg, Ca and P in DCAD does not result in a better explanation of urine pH. Urine pH seems to be estimated best from dietary content of Na, K, Cl and S, which agrees with the findings of Tucker et al. (1991).

In correspondence with the results obtained with DCAD, also with estimates of UCAD a more pronounced relationship was obtained between urine pH and UCAD when S was included (Figure 5B vs. 5A). Compared to DCAD, the relation was less clear however (Figure 5B vs. 4B). Because digestibility and excretion with milk were taken into account in estimating UCAD, a similar relationship was found when Mg, Ca and P were included, in contrast to the results obtained with DCAD. Therefore, the digestibility and the excretion of Mg, Ca and P in particular need to be taken into account if they are to be used in the calculation of cation anion difference. Because the relationship hardly changed by the inclusion of Mg, Ca and P (Figure 5C vs. 5B), it is concluded that Mg, Ca and P are less important determinants of urine pH for the data considered in the present study, or that estimates of digestibility and excretion rate were too inaccurate to clarify the relationship any further.

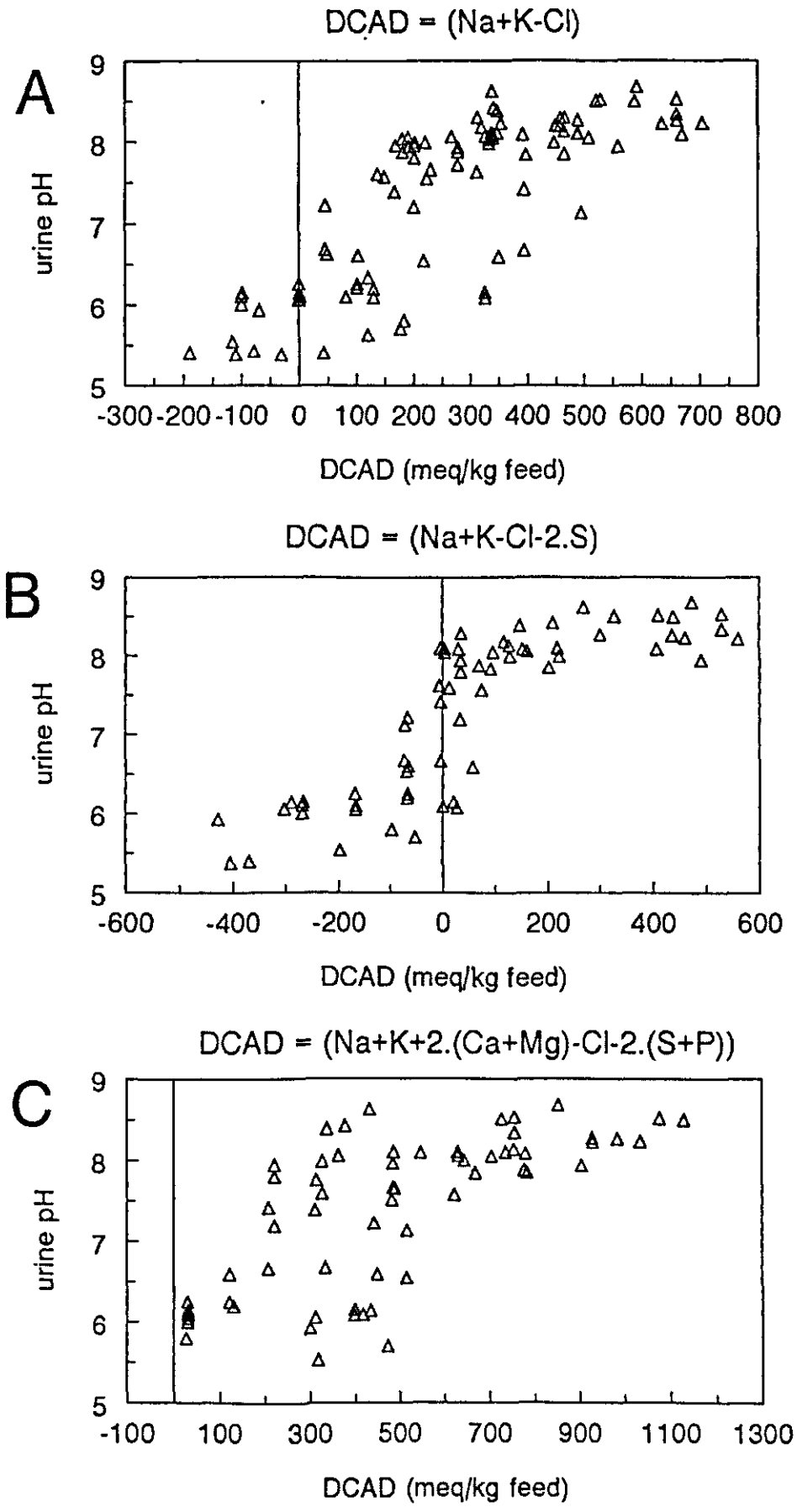


Figure 4. Relationship between urine pH and dietary cation anion difference (DCAD) for lactating and nonlactating dairy cows. The DCAD value was defined as A. (Na + K-Cl), B. (Na + K-Cl-2.S) and C. (Na + K + 2.Mg + 2.Ca-Cl-2.S-2.P).

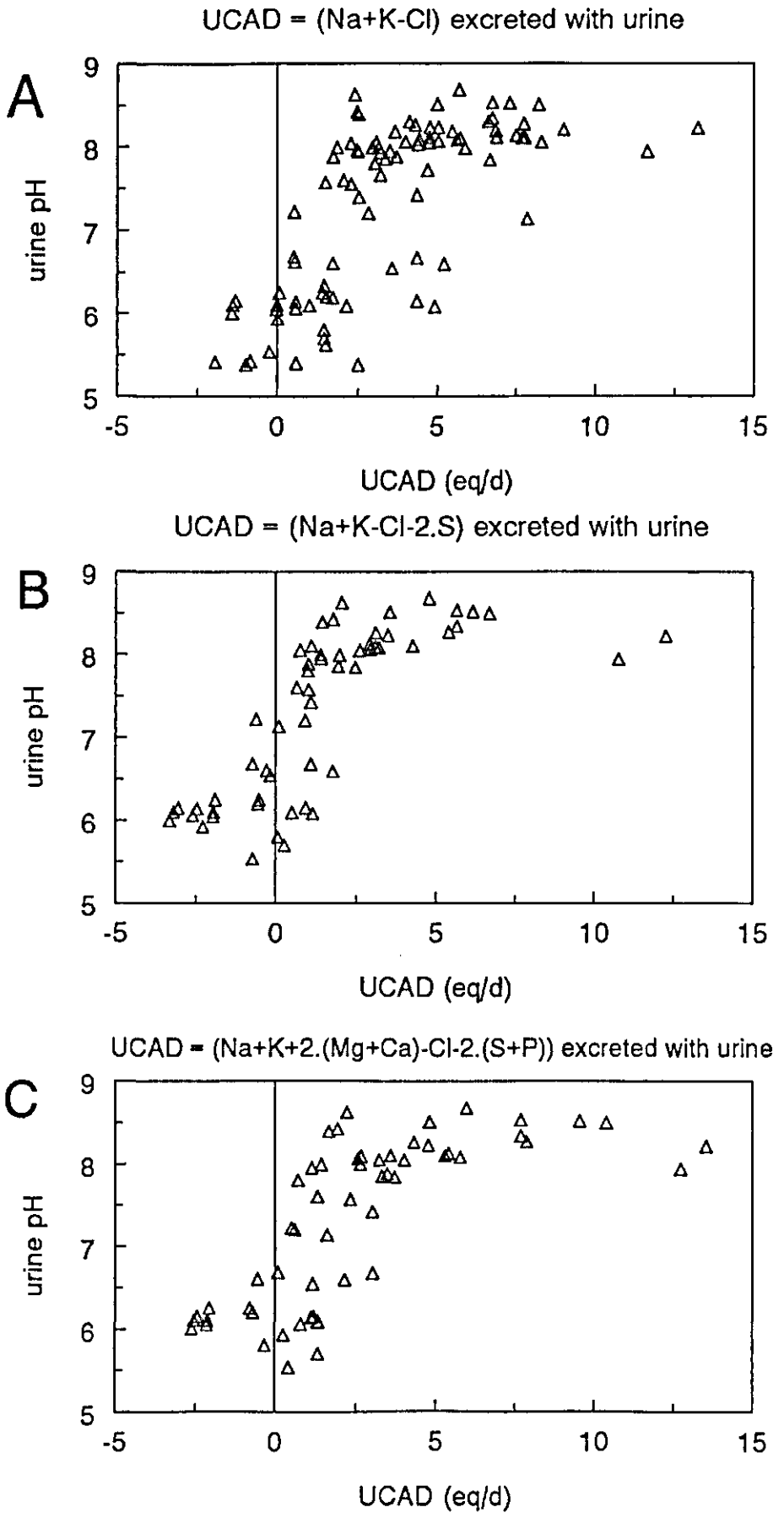


Figure 5. Relationship between urine pH and the estimated cation anion difference excreted with urine by lactating and nonlactating dairy cows. The UCAD value was defined as A. (Na + K-Cl), B. (Na + K-Cl-2.S) and C. (Na + K + 2.Mg + 2.Ca-Cl-2.S-2.P).

Implications of DCAD on ammonia emission

The first step in the process of ammonia emission is the hydrolysis of urea, which is in most circumstances the main nitrogenous component present in urine of dairy cows, to ammonia by the enzyme urease. Urease activity is high on surfaces where faeces or urine is deposited regularly (Monteny, 1996). In a scale model of a dairy-cow house, Elzing & Monteny (1997b) demonstrated that ammonia emission is linearly related to the urea concentration in the urine sprinkled. Hence, a reduced urease activity by low pH values will effect the rate of urea conversion into ammonia. A urine pH below 7 decreases urease activity, which is thought to become almost absent at pH 5.5 (van Vuuren & Jongbloed, 1994). But, values of urine pH this low are not occurring for long periods in the current practise of feeding dairy cows. Also, more quantitative information seems to be needed on how urease activity varies under various conditions. Elzing & Monteny (1997a) modified the Michaelis-Menten kinetics established from their previous laboratory work to obtain a good fit between observed and predicted ammonia emission rates that were established with the scale model. Notwithstanding the potential effect of a low pH on urease activity, the ammonia concentration is thought to be the most important factor in determining ammonia emission from floors, in addition to physical factors like temperature and ventilation rate (Monteny, 1996). The emission of ammonia from urine puddles on the floor is generally considered to be linearly dependant on the ammonia concentration (Muck & Steenhuis, 1981; Aarnink, 1997; Elzing & Monteny, 1997b) and has been modelled by the following equation:

[7] $$E = (k \times A \times [C] \times f) / H$$

where

- A = the emitting surface area (m^2)
- [C] = the sum of the ammonium and ammonia concentration ($mol.m^{-3}$)
- k = the mass transfer coefficient ($m.s^{-1}$)
- f = the ammonia fraction in [C]
- H = Henry's constant

Nutritional measures to lower urine pH will affect the ammonia fraction in the urine according to the Henderson-Hasselbalch equation. A reduction of urine pH from 8.5 to 7.0 will shift the ammonia fraction from 0.182 to 0.006. An empirical equation was derived by Hashimoto & Ludington (1971) from observation on concentrated chicken manure slurry with high ammonia concentrations, including the effect of temperature. They found that the dissociation constant for ammonia was one-sixth of that found in water. Their empirical equation predicts a shift of the ammonia fraction from 0.025 to 0.001 at 20°C. Because of the linear dependance between ammonia emission and the ammonia concentration in eq.7, a lowering of pH from 8.5 to 7.0 is expected to cause a 25-fold reduction in ammonia emission rate, whereas a further reduction to 6.0 will result in even a 250-fold reduction. The reduction actually realised will be much smaller though, because the pH of acid urine puddles on the floor surface rapidly increases after urination. This increase in pH is caused by a high ammonia concentration generated by the hydrolysis of urea to ammonia and carbon dioxyde, and because of a more rapid diffusion of carbon dioxyde than ammonia from the fluid layer to air (Aarnink, 1997). Although the increase in pH after urination has already been established, it

would be interesting to investigate how this increase is affected by altered chemical and physical characteristics of urine puddles (for example because of a changed DCAD). In order to precisely quantify the effect of urine pH on ammonia emission from floors, accurate information is needed on the factors that determine the pH value in puddles of urine on the floor surface, like UCAD and the buffering capacity of urine (Van Vuuren & Jongbloed, 1994). Van Vuuren et al. (1996) and De Jong (1996) demonstrated a significant influence of nutrition on the buffering capacity of urine. However, it must be kept in mind that a manipulation of DCAD can only result in a substantial effect on urine pH in the range of +100 to -100 meq/kg of feed DM. Forages in dairy cow rations often have a high K content and therefore highly positive DCAD values (Block, 1994). Then the addition of acidifying salts for example will have little effect on urine pH.

Finally, a chronic metabolic acidosis induced by nutritional measures can cause some metabolic changes in the animal. Of importance to ammonia emission is the reduced ureagenesis in the liver and the increased ammoniagenesis in the kidney which has been well established (Meijer et al., 1990; Meijer et al., 1993; Welbourne, 1987). In general, an increased amount of ammonium is excreted with urine by dairy cows fed diets low in DCAD (Delaquis & Block, 1995; Schonewille et al., 1994; Van Mosel et al., 1993; Wang & Beede, 1992a&b; Kilmer et al., 1982). However, urine pH did not fully explain the reported amounts of ammonium excreted. Further, the increase in ammonium excretion is compensated by a decrease in urea excretion, and has probably a small net effect on the sum of urea N and ammonia N excreted. A change in urine pH will affect ammonia emission mainly by changing the ammonia fraction in urine puddles, leaving metabolic changes as less important factors.

Effects of DCAD on animal health

The requirement of diary cows for Ca increases substantially around parturition with the onset of the excretion of the colostrum (Van Mosel, 1991). If during this stage the onset of Ca mobilization develops insufficiently or too slowly, milk fever can develop with a severe decline of the Ca content in blood. In order to make parturient cows less susceptible to milk fever, the mobilization of Ca is stimulated by changing the diet several weeks before parturition. First, the Ca content in the diet is kept low to stimulate the efficiency of Ca absorption and to allow more Ca to be absorbed with the onset of milk production at parturition. Because a low Ca content is difficult to accomplish with most forages, Ca intake prepartum is lowered by reducing dry matter intake (Van Mosel, 1991). Second, there are indications that the incidence of milk fever is lowered by changing the DCAD of the diet from positive to negative before parturition (Block, 1984). A negative DCAD has an acidifying effect on acid-base equilibrium (Stewart, 1983), resulting in acidic urine, in a stimulation of the mobilization of Ca from bone, and in an increased urinary excretion of Ca (Van Mosel, 1991; Schonewille, 1994a&b).

The scope of this study was to investigate the possibility to reduce ammonia emission by lowering urine pH. In this context, the question arises how a prolonged lowering of DCAD, in order to reduce urine pH, will affect animal health. Schonewille et al. (1994a) argued that Ca excreted with urine increases with a further decrease of negative DCAD values. Such an increase in Ca loss will only partly be compensated by an increased efficiency of intestinal

absorption. Because under such conditions an increase in Ca intake will down-regulate the efficiency of intestinal absorption (Schonewille et al., 1994a), these Ca losses can not be compensated by an increased Ca intake. Part of the Ca excreted with urine is delivered by a net resorption of bone. Van Mosel (1991) established that Ca mobilization from bone is accomplished by a reduced bone accretion rate. Schonewille et al. (1994a&b) reported 1 to 5 g of Ca loss/d with DCAD ranging from -170 to -114 meq/kg of feed. Considering such amounts of Ca loss, it seems possible to feed dairy cows a diet with negative DCAD values for prolonged periods (at least months) without serious consequences for bone condition. Moreover, the disturbing effects on Ca homeostasis will be more gradual in comparison to the sudden increase in Ca loss with the onset of the production of colostrum. Gradual changes in Ca mobilization and Ca balance possibly better allow homeostatic mechanisms to regulate the capacity to absorb Ca from the gastrointestinal tract, bone metabolism, and the capacity of Ca resorption by the kidney, in order to restore Ca balance in the long term. Besides the potential effects on bone metabolism and Ca homeostasis, also other health problems may be caused by a metabolic acidosis. No information was found in literature in relation to long term feeding of diets negative in DCAD to dairy cows. The consequence on animal health and animal performance needs to be established in feeding trials before these diets can be applied in practise.

Besides the addition of salts, mineral acids added to conserve feedstuffs or prepare silages also results in low DCAD values and can induce a metabolic acidosis and acidic urine (Brouwer, 1934). Further, feeding extreme amounts of rapidly fermentable carbohydrates causes a reduction of rumen pH and in extreme cases an accumulation of lactic acid in the rumen (Counotte, 1981). Absorption of considerable quantities of lactic acid to systemic blood then causes a metabolic acidosis as well. Although all these measures may accomplish a low urine pH, feeding extreme amounts of rapidly fermentable carbohydrates and possibly the addition of mineral acids (Brouwer, 1934) is not practicable because of potential health problems or a limiting effect on microbial activity, rumen digestion, feed intake and animal performance.

5. Conclusion

It is possible to reduce the pH value of urine excreted by dairy cows by a lowering of DCAD, irrespective of the physiological state of the animals. A change in urine pH of about two and a half units is possible, which will cause a 250-fold decrease of the ammonia fraction present in urine or in urine puddles on the floor immediately after urination. Calculation of DCAD by taking into account Na, K, Cl and S appears to give the best explanation of the pH values found in urine of dairy cows. Corresponding to acid-base theory, a change of DCAD will have the largest effect on urine pH near DCAD values of 0 meq/kg of feed DM. Further research is needed on the consequences of chronic administration of low DCAD diets on animal health and performance. For an accurate prediction of the effect of urine pH on ammonia emission, it seems worthwhile to investigate what characteristics of urine and faeces affect the chemical and physical factors that determine the emission rate of ammonia.

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