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Concentrate feeding and ruminal fermentation. 3. Influence of concentrate ingredients on pH, on DL-lactic acid concentration in rumen fluid of dairy cows and on dry matter intake

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Summary

Several concentrate ingredients were administered daily at 09h00 into the rumen of 4 cows via a rumen cannula over 5 successive days. Between 09h00 and 13h00, samples of rumen fluid were taken frequently to measure pH and D- and L-lactate concentration.

Good-quality hay was given ad libitum at 13h00 each day when a concentrate ingredient was administered. Between experimental periods, hay was given twice a day at 08h00 and 17h00. Length of these intervals was 17 days at least. Hay refusals were weighed every day at 07h30. From the same batches of concentrate ingredients also 6-kg amounts of 1:1 mixtures of two ingredients were dosed. In concentrate ingredients content of glucose, fructose, sucrose, soluble α-glucose-linked carbohydrates, total starch and soluble protein was analysed.

pH minima and D- and L-lactate concentration maxima in rumen fluid were reached between 2 and 3 hours after administering. Lowest pH and highest D+L-lactate concentration in rumen fluid relative to the situation after administering of citrus pulp – taken together as the potential risk of lactic acid acidosis (RLA) – was best correlated with the content of glucose + fructose (GF) and soluble protein (SPROT); RLA = 1.47 GF + 0.49 SPROT + 22.50 (r = 0.89)

Arrangement of the single feedstuffs according to their potential RLA (based on the results of the present experiments) agreed fairly well with the index based on experiments in vitro with feedstuffs of the same batches (Malestein et al., 1982).

When combined feedstuffs were administered, pH and D+L-lactate concentrations in rumen fluid differed from values calculated from results after giving the same feedstuffs separately.

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Introduction

In order to meet the energy demands of high-producing dairy cows, after parturition the rations are changed normally from a ration consisting of roughage only or with a minor proportion of concentrates to a ration with a large proportion of concentrates. When this change-over occurs too rapidly, large quantities of DL-lactate are produced by the rumen bacteria and protozoa. The increase in concentration of DL-lactate in the rumen strongly depends on the amount of feed and the nature of the feedstuff components ingested (Counotte, 1981).

In incubations in vitro of rumen fluid with several feedstuffs, normally used to compose concentrate mixtures for ruminants, the changes in pH and in lactic acid concentration during 4 to 6 h of incubation showed clear differences between feedstuffs (Malestein et al., 1982). However, in the rumen not only fermentation processes play a role. For example, lactic acid disappears from the rumen during passage to the lower gut and by absorption (Counotte & Prins, 1979). Moreover, it is shown that after repeated feeding microbial adaptation (Mackie et al., 1978; Fulton et al., 1979; Counotte, 1981) affects changes in pH and DL-lactic acid concentration from day to day. We decided, therefore, to investigate whether the acidotic index of feedstuffs based on incubations in vitro could be affirmed in vivo. If so, then the potential risk of lactic acid accumulation in the rumen could be predicted from the results obtained with incubations in vitro, and the method in vitro would be suitable as an 'acidosis test' for feed ingredients.

In the present experiments the effect of (single) concentrate feedstuffs on the change in pH and on lactic acid concentration in rumen fluid was studied after administering daily 6-kg amounts of these feedstuffs into the rumen of dairy cows. The pH decrease and lactic acid concentration increase were related to the content of starch, soluble carbohydrates and soluble protein. Feedstuffs were from the same batches as used in our previous experiments in vitro (Malestein et al., 1982).

Material and methods

The experiments were carried out with 4 milk-producing Friesian × Holstein cows (body weight about 600 kg) fitted with rumen cannulae (ø 5 cm). Of each of seven feedstuffs (tapioca, maize meal, solvent-extracted soya bean meal, coconut meal (expeller), beet pulp, maize gluten feed meal and citrus pulp) 6-kg amounts were administered into the rumen of each cow via a rumen cannula. The sequence of feedstuff administration into the rumen of the 4 cows is given in Table 1. Administering via the rumen cannulae was done to avoid differences in amount consumed or in rate of intake between the feedstuffs which would influence the results. This procedure of dosing required a maximum time of 6 minutes each time (6 kg). The amount of 6 kg of concentrates was chosen since it was found in preliminary experiments that clear effects on pH and lactic acid concentration in rumen fluid could be expected with such quantities without seriously disturbing rumen function. Moreover, this amount agreed with the dosing of 1 g per 20 ml of diluted (50 % v/v) rumen fluid as used in earlier experiments in vitro (Malestein et al., 1982) if it is as-

Table 1. Sequence of ingredient administration per cow and per period.

Cow	Period I	II	III	IV	V	VI	VII
N 240	beet pulp	maize meal	coconut meal	maize gluten feed	citrus pulp	tapioca	soya bean meal
N 248	maize meal	beet pulp	maize gluten feed	tapioca	coconut meal	soya bean meal	citrus pulp
S 218	citrus pulp	soya bean meal	tapioca	maize meal	beet pulp	coconut meal	maize gluten feed
I 332	tapioca	citrus pulp	beet pulp	soya bean meal	maize meal	maize gluten feed	coconut meal

sumed that the rumen of the cows contain approximately 60 l of fluid. Administering was done during 5 successive days at 09h00. From 09h00 to 13h00 samples of rumen fluid were taken every 15 minutes (Ventral rumen sac) for estimating pH and DL-lactic acid concentration. pH was measured immediately after sampling. Subsamples (1 ml) were taken for the analysis of D- and L-lactic acid and put into tubes containing 1 ml $ZnSO_4$ (5 % w/v). D- and L-lactic acid concentrations were analysed enzymatically (Bergmeyer, 1970).

When concentrates are fed with a high content of easily fermentable components the risk of rumen dysfunction and of lactic acid acidosis increases, according to the literature, with the decrease of the pH and the increase of the lactic acid concentration in rumen fluid. In the present experiments the lowest pH and the highest lactate concentration was always found when citrus pulp was administered into the rumen, i.e. the potential risk of lactic acid acidosis (RLA) was highest when citrus pulp was given. The RLA of the other feedstuffs investigated was expressed relative to the RLA of citrus pulp as follows. When the pH decrease and the increase of lactic acid concentration in rumen fluid after the administration of a feedstuff into the rumen were 70 % and 60 % of the values found for citrus pulp respectively, the RLA of the feedstuff was put at 65.

The pH decrease was calculated as the difference between 6.63, the mean pH measured on 9h00 on the first day, and the lowest pH measured on the day with the highest potential risk.

Good-quality hay was supplied ad libitum at 13h00. Between each 5-day experimental period (Table 1) an interval period of at least 17 days was taken. During this interval period the cows were allowed to eat good-quality hay ad libitum. Refusals of the hay were gathered daily and weighed at 07h30.

After the experiments in which single ingredients were given, 2 cows were given mixtures of two feedstuffs during 3 days. In these experiments the conditions were the same as when the single ingredients were given. Cow N240 was given 6 kg of maize/maize gluten feed (50/50) and cow S218 was given 6 kg of citrus pulp/soya bean meal (50/50). Concentrate ingredients used in all these experiments were from the same batches as used in the foregoing experiments in vitro (Malestein et al., 1982).

Table 2. Content of soluble sugars and soluble protein of the different feedstuffs (g/kg dry matter).

	G + F[1]	SCARB[2]	TSTAR[3]	SPROT[4]
Maize	3	15	616	4
Tapioca	10	1	706	2
Beet pulp	6	72	12	24
Citrus pulp	44	52	51	29
Maize gluten feed	3	9	225	73
Coconut expeller meal	12	63	8	20
Soya bean meal solvent extracted	1	67	16	85

[1] Glucose + fructose.
[2] Sucrose + soluble α-glucose-linked carbohydrates.
[3] Total starch.
[4] Protein soluble in rumen fluid.

Analysis of sugars, soluble starch and soluble protein
Feedstuffs were ground to pass a 2-mm sieve and suspended (5 %, w/v) in sterile rumen fluid for 1 hour at 39 °C. After centrifugation at 4 °C, the supernatant was analysed for soluble sugars and protein content. Total soluble carbohydrate content of the feedstuffs was determined with the anthron method (Herbert et al., 1971). Glucose, fructose, sucrose and α-glucose-linked carbohydrates were determined enzymatically (Bergmeyer, 1970). Soluble protein was determined after Kjeldahl (Herbert et al., 1971). Results of these analysis are recorded in Table 2.

Results

pH and DL-lactate after giving single ingredients
Administration of 6-kg amounts of tapioca, maize meal, soya bean meal, coconut meal, beet pulp, maize gluten feed or citrus pulp into the rumen of fistulated cows resulted in a decrease of the pH and in many cases in an increase of the lactic acid concentration of rumen fluid. Fig. 1 shows the changes in pH and L-lactic acid concentration in rumen fluid after administering beet pulp for the 5 days of the experimental periods and for each of the 4 cows separately. In most cases the lowest pH was reached between 2 and 3 hours after giving beet pulp while the highest lactic acid concentrations were seen somewhat earlier.

Immediately before feeding (09h00), the pH in rumen fluid was lower on the 2nd and, less so, on the 3rd day compared with the other days. This shows instability of rumen fermentation. Within feedstuff animals between differences were noticed in the level of the pH. In the trials with beet pulp the pH of rumen fluid of cow N 240 and S 218 decreased less than in cow N 248 and I 332.

Further it appears from Fig. 1 that L-lactic acid concentrations were only slightly elevated on the 1st and 5th day. On the 4th day maximum values of 3 to 4 mmol/l were measured. The results obtained with the 4 cows differed not only with respect to the highest level of L-lactic acid reached, but also with regard to the day on which the highest concentrations were estimated.

Comparable trends as shown after dosing beet pulp during 5 days were noticed

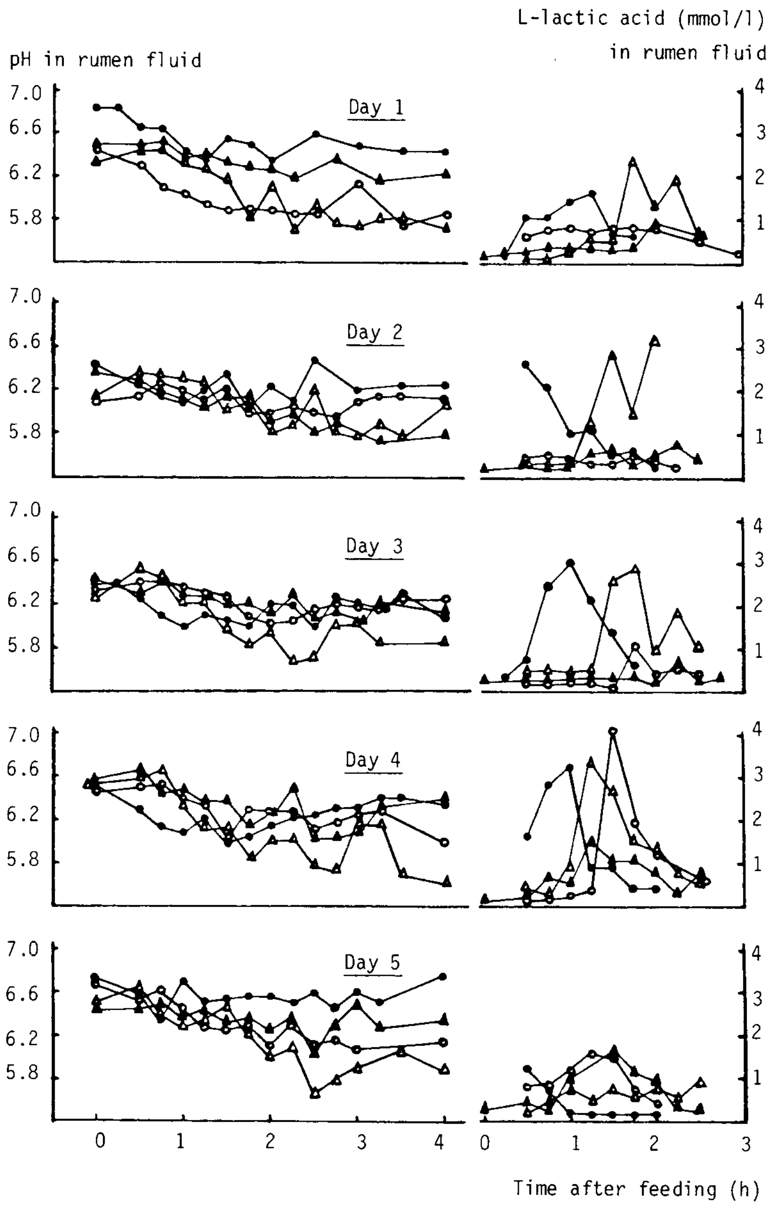

Fig. 1. Rumen pH and L-lactic acid concentration (mmol/l) in four cows (N 240 (●), N 248 (○), I 332 (△), S 218 (▲)) after giving of 6 kg of beet pulp during 5 days.

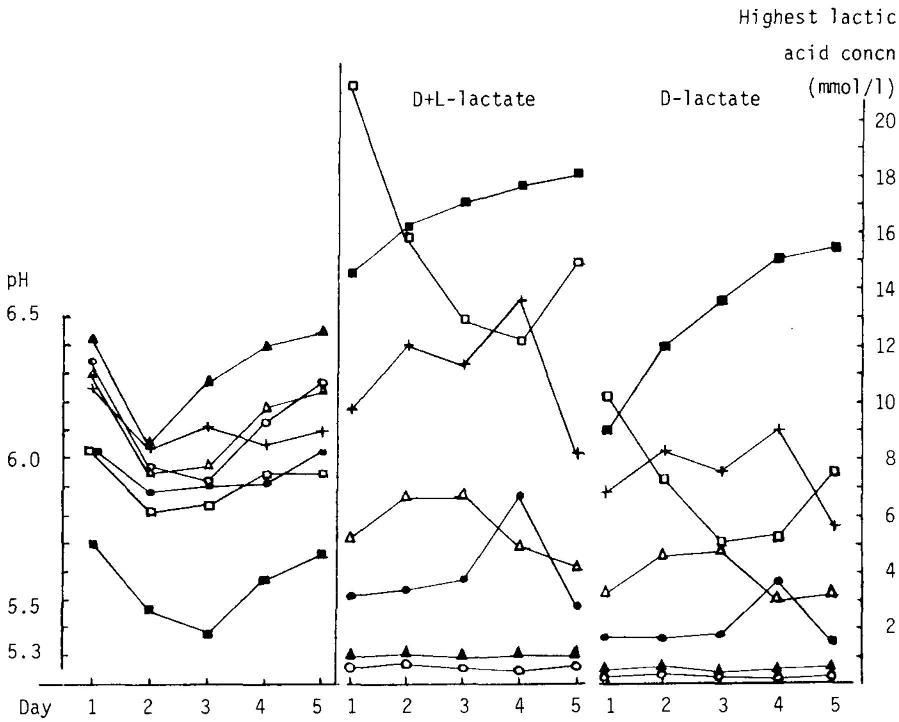

Fig. 2. Daily lowest pH and highest lactic acid concentration (mmol/l) (D and D+L) in rumen fluid during five days of giving 6 kg of tapioca (▲), maize meal (○), soya bean meal (△), coconut meal (+), beet pulp (●), maize gluten feed (□) or citrus pulp (■). Mean of 4 cows.

with the other 6 feedstuffs. In Fig. 2 results of measured pH values (daily lowest values reached) and of the D+L-lactic acid concentrations (daily highest values analysed) on 5 successive days are summarized (mean of 4 cows). From Fig. 2 it appears that on the first day the mean lowest pH differed very clearly between feedstuffs. On the second day, pH was lower with all feedstuffs. On the third day the pH decreased less except after dosing of maize meal and citrus pulp. On the 5th day the pH level per feedstuff agreed with the level on the first day (except for coconut meal).

Great differences were found between feedstuffs on the first day in total lactic acid concentration (sum of D- and L-lactate). High values were analysed after administration of maize gluten feed (approximately 21 mmol/l), while low values were analysed after administration of maize meal (approximately 0.6 mmol/l) and tapioca (approximately 0.8 mmol/l). The following days total lactate concentrations remained low after giving maize meal and tapioca, declined after giving maize gluten feed until the 4th day, fluctuated somewhat after giving beet pulp, soya bean meal and coconut expeller meal and increased with citrus pulp.

Finally, it can be deduced from Fig. 2 that the ratio between D- and L-lactate

(D/L ratio) was not constant but varied between feedstuffs and between days. When maize meal, tapioca or beet pulp was fed the D/L ratio was approximately 1. This also was the case with maize gluten feed, but with this feedstuff the D/L ratio became wider until the 3rd day (D/L ratio 1.6) and narrowed after that day. When soya bean meal or coconut meal was given the D/L ratio always was approximately 2.5. For citrus pulp the initial D/L ratio was 2.5, but the following days the proportion of D-lactate increased strongly, which resulted in a D/L ratio of 5.5.

pH and DL-lactate after giving mixed ingredients

Analogous to the results obtained with single ingredients the results obtained after administering mixed ingredients are given in Fig. 3. In Fig. 3, pH values and L- and D-lactate concentrations in rumen fluid after giving mixed ingredients are given together with the calculated values obtained after giving the ingredients separately to the same cow. Measured pH minima were lower than calculated, probably because the influence of the single ingredients on pH decrease and on lactic acid concentration increase was additive. Measured values of L-lactate concentrations generally were equal to or lower than those calculated and measured D-lactate concentrations did not agree fully with calculated values (Fig. 3). After administering a mix-

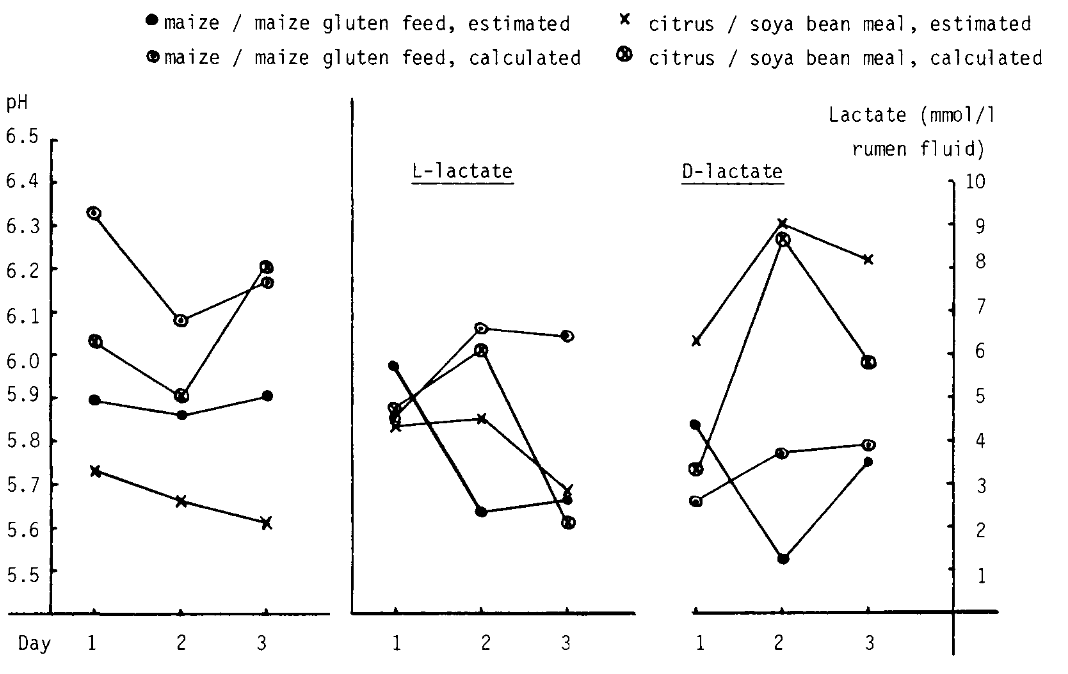

Fig. 3. Daily lowest pH and highest lactic acid concentration (mmol/l, D and L) in rumen fluid during three days of giving 6 kg maize/maize gluten feed (50/50) (●) or citrus pulp/soya bean meal (50/50) (×) mixtures. Values calculated from giving maize and maize gluten feed separately (⊙) or citrus pulp and soya bean meal separately (⊗) are given as well.

ture of maize/maize gluten feed the D/L ratio varied from 0.5 to 1.3 and after giving the citrus pulp/soya bean meal mixture the D/L ratio increased from 1.4 to 2.9.

Dry matter intake

Daily dry matter intake from hay during 4 days prior to the first day of administration of a feedstuff into the rumen and during the days of giving single ingredients is given in Fig. 4. From this figure it appeared that prior to administering, daily dry matter intake from hay varied from 13 to 15 kg (mean of 4 cows). As was to be expected dry matter intake from hay decreased during days of giving 6-kg amounts of concentrates. This decrease was smallest when maize gluten feed was given and highest when soya bean meal was given. After 3 or 4 days of giving the ingredients hay intake increased. In a few instances hay refusals were not weighed on the 5th day of administration and therefore hay intake could not be given in Fig. 4. In other cases where the refused hay was always measured it could be calculated that hay intake on the 5th day was higher than on the 4th day.

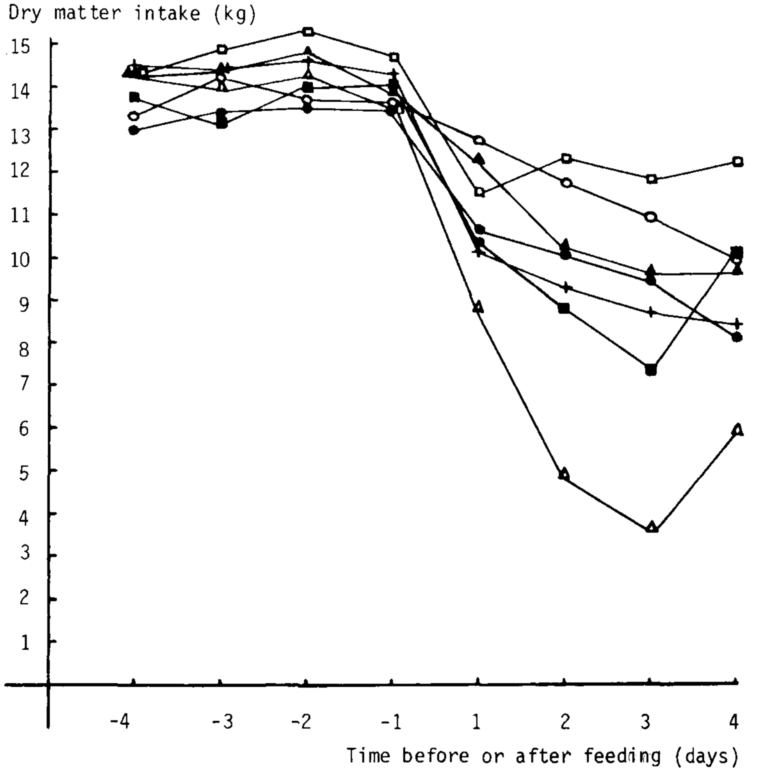

Fig. 4. Daily dry matter intake from hay before and during periods of giving 6 kg tapioca (▲), maize meal (○), soya bean meal (△), coconut meal (+), beet pulp (●), maize gluten feed (□) or citrus pulp (■). Mean of four cows.

Relationship between carbohydrate fractions and soluble protein and the risk of lactic acid acidosis

When the 7 ingredients used in this experiment were ranked with respect to their effects on lactic acid accumulation and decrease in pH in rumen fluid (taken as the potential risk of lactic acidosis (RLA) relative to the values for the feedstuff with the highest RLA, i.e. citrus pulp), the following sequence appeared: citrus pulp (100), maize gluten feed (80), coconut expeller meal (60), solvent-extracted soya bean meal (48), beet pulp (48), maize meal (28) and tapioca (26). The lowest pH and the highest lactic acid concentration measured in the rumen after giving these feedstuffs, relative to the values observed after giving citrus pulp (RLA), were correlated with the content of glucose + fructose (GF), soluble carbohydrates, total starch or soluble protein (SPROT). The potential risk of acidosis in vivo showed to be best correlated with the sum of the glucose + fructose and soluble protein content of the feedstuffs ($P \leqslant 0.05$). The following regression formula was obtained: RLA = 1.47 GF + 0.49 SPROT + 22.50 ($r = 0.89$; F = 7.79).

RLA was negatively correlated with the total starch content of the feedstuffs. The role of soluble proteins as an contributing factor to DL-lactate accumulation is striking since DL-lactic acid is not a major product of protein fermentation.

Discussion

An excessive ingestion of feedstuffs rich in readily fermentable substrates gives rise to an increased acid production and an accumulation of DL-lactate in the rumen. Feed components that are claimed to bring about lactic acid acidosis are starch and sugars (Dunlop, 1972; Slyter, 1976; de Visser, 1982). From the experiments described by Byers & Goodall (1979) and by Counotte (1981) it can be concluded that feeding starch results in high DL-lactic acid production rates only when the diet contains very high levels of this carbohydrate, i.e. rations with a high grain proportion. In the Netherlands the concentrate mixtures for ruminants contain many different feedstuffs among which wastes of industrial food products and tropical plants residues. These concentrates may contain considerable proportions of soluble sugars such as glucose, fructose and sucrose which are fermented faster than starch and are thus likely to stimulate the DL-lactate production much more than starch. For example, with tapioca and maize meal 3.5 kg and 3.0 kg starch was given respectively, but high lactic acid levels did not appear.

The significant contribution of the soluble protein in the feedstuffs to the RLA or to the DL-lactate accumulation in the present experiments can be explained by the stimulating effect of protein on the growth rate of DL-lactic acid producing organisms (Counotte, 1981). These organisms produce lactic acid only at high growth rates. Higher growth rates result in higher glycolytic fluxes and therefore in a percentual higher DL-lactic acid production.

D/L ratio

Accumulation of D-lactate in the blood especially is thought to be responsible for the acidosis which ultimately may result in the death of the animal (Dunlop, 1972).

Therefore, besides the concentration of DL-lactate, the D-lactate concentration or the D/L-lactate ratio deserves special attention.

In sheep Giesecke et al.(1976) found that during the change from a hay ration to a ration of hay and maize (1:3) the D/L ratio increased strongly when pH in rumen fluid reached lower values. In preceding experiments we found a D/L ratio of 2.5 to 3 when feeding maize-soya bean meal concentrates to adapted cows, with no influence of pH on this ratio (Malestein et al., 1981). In the present experiments again no pH effect on D/L ratio could be ascertained, but this ratio was determined by the feedstuff supplied. Also Giesecke et al. (1976) found differences in the D/L ratio when feeding different feedstuffs. When glucose was fed the D/L ratio was approximately 2.7, while after feeding a same amount of fructose or of sucrose this ratio was approximately 1. Giesecke & Bartelmus (1972) found a D/L ratio of 1 when feeding a maize ration, and this ratio was independent of the pH. When administering maize meal into the rumen we also found a D/L ratio of 1. On the other hand with citrus pulp the D/L ratio increased from 2.5 (1st day) to 5.5 (5th day) and with the citrus pulp/soya bean meal mixture the D/L ratio increased to 2.9 (3rd day). Therefore, the risk of rumen lactic acidosis may be increased with concentrates rich in ingredients which give a high D/L ratio (for example citrus pulp).

After feeding straight into the rumen, the highest value of D-lactate concentration in the rumen coincided with the highest L-lactate concentration. At that time also the D/L ratio reached its highest value. This finding agreed with that of Mackie et al. (1978) in experiments with sheep and with that of Malestein et al. (1981) in experiments with cows in which the influence of frequency of feeding concentrate mixtures on rumen pH was studied. Based on these data and on those of Giesecke et al. (1972, 1976) it appeared that the D/L ratio in rumen fluid will be determined strongly by the composition of the ration and by the time of sampling after feeding, whether D- and L-lactate concentrations are at a maximum level or not.

In our experiments in vitro (Malestein et al., 1982) with feedstuffs of the same batches as in the present experiments, the D/L ratio never exceeded 0.3. The much higher ratios found in vivo indicate that factors other than feedstuffs and time after sampling affect the D/L ratio.

Adaptation

In the present experiments after a sudden change from a hay ration to a ration consisting of hay and 6 kg of a concentrate ingredient, the lowest pH values in rumen fluid were measured on the 2nd or 3rd day after the change. Giesecke et al. (1976) also found the lowest pH values in rumen fluid on the 3rd day after a sudden change from a hay ration to a ration of hay and maize meal (1:3). Sutton & Johnson (1969) also found lowest values in pH of rumen fluid 3 to 4 days after a sudden change from hay to flaked maize or to rolled barley. The lowest value found after feeding flaked maize was lower than after feeding rolled barley and the trend in pH from 3 days after the change onwards was different between the feedstuffs. Although in our experiments the change from hay to hay with 6 kg of concentrates was less abrupt than in the experiment of Sutton & Johnson (1969), pH in rumen fluid (lowest values) also reached highly varying levels between the different feedstuffs. Moreover, after reaching the minimum pH values (2nd or 3rd day) upon feeding tapioca or maize meal the recovery of pH was faster than when coconut meal or beet pulp were fed.

Four hours after feeding flaked maize, Sutton & Johnson (1969) found higher lactic acid concentrations than after feeding rolled barley. In our experiments, 4 h after administering the ingredients lactic acid concentrations always had decreased to very low levels even after reaching maximum values of 20 mmol/l. In addition, differences in rate of dry matter intake between the feedstuffs may be of influence on lactic acid levels. When feeding maize meal Fulton et al. (1979) found that the initial rate of intake was the same as when feeding wheat meal. After some days the rate of intake of maize meal was higher than with wheat and this coincided with higher lactic acid concentrations after feeding maize meal. When the proportion of concentrates in the ration increased, the rate of intake decreased which coincided also with lower lactic acid concentrations in rumen fluid. In our experiments all concentrates were given via the cannulae during almost the same period of time (6 minutes per feeding of 6 kg of concentrates) so that differences in pH or in lactic acid concentrations were not the result of differences in rate of intake neither from day to day nor between feedstuffs.

After a sudden change from a hay ration to a mixed hay-concentrate ration in experiments of Fulton et al. (1979), dry matter intake decreased until the 3rd day and then increased again. When feeding the ration with wheat meal, the decrease in dry matter intake was larger than with maize meal, and the rumen pH was considered as the causative factor. In our experiments roughage (hay) was supplied separate from the concentrates. The decrease in hay intake was largest with soya bean meal and lowest with maize gluten feed. Daily lowest pH value in rumen fluid however was lower after maize gluten feed than after soya bean meal. Likewise after giving citrus pulp, pH in rumen fluid reached lower values than after giving soya bean meal, but intake of hay remained higher in the period in which citrus pulp was given. Consequently differences in hay intake when concentrate-rich rations were fed cannot be explained by differences in rumen pH. However within concentrates the daily lowest pH measured was correlated positively with the dry matter intake from hay.

In vitro versus in vivo experiments

When the single feedstuffs used in the earlier experiments in vitro (Malestein et al., 1982) and those of the present experiments in vivo were arranged according to lowest pH value and highest lactic acid concentration measured in rumen fluid, there appeared a fairly good agreement (Table 3). The agreement in rank was better when based on pH than on lactic acid. Soya bean meal incubations in vitro resulted in very high lactate concentrations. This was not the case in vivo.

In incubations in vitro with combined ingredients (Malestein et al., 1982) the pH decrease and the lactic acid accumulation in most cases did not agree with the values calculated from the incubations with single feedstuffs assuming additivity of the effects. The results of the present experiments in vivo confirmed that the risk of lactic acid acidosis deduced from experiments with single feedstuffs cannot be used to predict the RLA for concentrate mixtures. However, it is not excluded that the

Table 3. Values and rank of L- or D + L-lactic acid concentration (mmol/l) and of pH in rumen fluid in vitro compared with experiments in vivo with the same feedstuffs.

	In vitro[1]					In vivo[2]			
	n	L-lactate		pH		D+L-lactate		pH	
		rank	mean ±S.D.	rank	mean ±S.D.	rank	mean ±S.D.	rank	mean ±S.D.
Tapioca	7	1	7.0 ± 5.1	1	5.81 ± 0.3	2	1.2 ± 0.4	1	6.08 ± 0.2
Coconut expeller meal	4	2	17.6 ± 7.7	2	5.43 ± 0.1	5	12.6 ± 6.1	2	6.00 ± 0.1
Maize meal	7	3	19.5 ± 5.8	4	5.24 ± 0.2	1	0.7 ± 0.1	3	6.00 ± 0.3
Maize gluten feed	5	4	23.0 ± 5.7	6	5.09 ± 0.1	6	15.9 ± 7.5	6	5.82 ± 0.2
Beet pulp	4	5	23.1 ± 7.9	5	5.18 ± 0.2	3	6.0 ± 1.3	5	5.95 ± 0.2
Citrus pulp	5	6	31.8 ± 4.5	7	5.04 ± 0.1	7	17.1 ± 5.7	7	5.39 ± 0.1
Soya bean meal solvent extracted	5	7	40.7 ± 10.0	3	5.25 ± 0.2	4	6.7 ± 6.5	4	5.94 ± 0.1

[1] Values after 4 hours of incubation (Malestein et al., 1982).
[2] Peak values of D+L-lactate conc. (mmol/l) and lowest pH values measured on the day with the highest potential risk (mean of 4 cows).

RLA for concentrate mixtures can be indicated from incubations in vitro with those concentrate mixtures. This, however, has not been studied so far.

The fact that during the experiments in vitro (Malestein et al., 1982) D-lactic acid never accumulated strongly, so that D/L ratio in most cases was about 0.2, is striking. For example, for citrus pulp in vitro D/L ratio was 0.1 while in the present experiment D/L ratio during the first day of the experimental period was 2.5 and on the last day D/L ratio was 5.5. This cannot be caused by differences in fermentation rate (Counotte, 1981), so a difference in rate of absorption between D- and L-lactic acid may be responsible.

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