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Concentrate feeding and ruminal fermentation. 2. Influence of concentrate ingredients on pH and on L-lactate concentration in incubations in vitro with rumen fluid.

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Summary

An investigation was made into the influences of concentrate ingredients on pH and on L-lactate concentration (acidotic index) in incubations in vitro of these ingredients with rumen fluid.

– Rumen fluid sampled before feeding from hay-fed cows was diluted (500 ml/litre) with an anaerobic salt solution. Of this fluid 20-ml amounts were added to variable quantities of feedstuffs (mostly 1 gram) in rubber-stoppered flasks incubated in a water-bath at 39 °C. For some hours pH and L- and D-lactic acid were estimated at hourly intervals. Reproducibility of this test after 5 hours of incubation was very high.

– After at least 4 hours of incubation in vitro of different feedstuffs strong differences in pH and in lactic acid concentration could be observed. Increasing the concentration of the substrates clearly influenced the acidotic index of the feedstuffs.

– Except with maize meal, hardly any effect of particle size on profiles of pH and of lactic acid concentration could be observed.

– Pelleting of a concentrate mixture hardly had any effect on profiles of pH and of lactate concentration as compared with no pelleting.

– Considerable differences in effect on pH and on lactic acid concentration were observed between different batches of the same feedstuffs especially with maize meal.

– When incubations were carried out with mixtures of concentrate ingredients pH and lactic acid concentration differed from values expected from results obtained with the single ingredients, assuming additive effects.

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– These experiments clearly show that the risk of lactic acidosis cannot be predicted from incubation in vitro with single ingredients.

Introduction

In rumen fluid of dairy cows higher lactate concentrations were measured after feeding concentrates containing approximately 230 g/kg starch plus sugars than after feeding concentrates containing approximately 500 g/kg starch plus sugars (Malestein et al., 1981).

This raised questions regarding the influence of concentrate ingredients on pH and on lactic acid production in the rumen. Literature concerning this subject is scanty and difficult to compare (Bath & Rook, 1963, 1965; Jensen, 1977; Lewis & McDonald, 1958; Sutton & Johnson, 1969; de Visser, 1980).

In the Netherlands concentrate mixtures are composed of a number of ingredients. Maize gluten feed, beet pulp, citrus pulp, soya bean meal and wheat bran are examples of such concentrate components. Abe & Kandatsu (1978) found a lower pH after 3 or 8 hours of incubation of maize starch with rumen contents in vitro than when either bran, maize meal or soya bean meal were taken as the substrate. With the nylon bag technique Nocek et al. (1979) showed faster degradation of wheat bran than, for example, of maize meal or soya bean meal. These data offered only limited information about the relationships between feed composition, DL-lactate concentrations in rumen fluid and the decrease in pH.

One of us (Prins, 1979) proposed to estimate the risk for rumen acidosis that concentrated feedstuffs may cause, when fed to ruminants, by way of an 'in vitro acidosis test'.

The experiments reported here concern a series of incubations in vitro in which single concentrate components were studied mainly with respect to their effect on pH and on lactic acid accumulation. In successive studies, to be published later, experiments were also carried out in vivo with concentrate components taken from the same batches as those used in this study.

Materials and methods

In vitro test

Cows fitted with rumen cannulae served as donors of rumen fluid. Their ration consisted of good-quality hay (approximately 260 g crude fibre per kg dry matter) fed ad libitum.

Rumen fluid, sampled just before feeding, was taken to the laboratory in a pre-warmed thermos flask and then once mixed (500 ml/litre) with an anaerobic salt solution (Hungate, 1966). In general the pH of the diluted rumen fluid was about 6.3, the temperature was kept at 39 °C. Of the fluid (referred to as rumen fluid in the following) 20-ml portions were added to variable quantities of a feedstuff in butyl rubber-stoppered flasks incubated in a water-bath at 39 °C. For several hours the pH was measured hourly and 1-ml subsamples were taken for analysis of DL-lactate. These values are used as an acidotic index. During all operations an anaerobic condition was maintained in the flasks by gassing with pure carbon dioxide.

D- and L-lactate were measured enzymatically (Bergmeyer, 1970). The reproducibility of the in vitro test after 5 hours of incubation with two feedstuffs was:
– tapioca n = 9, final pH 6.03 (range 6.00-6.05); L-lactate = 2.79 mmol/litre (range 0.74-5.18);
– citrus pulp n = 10, final pH 4.85 (range 4.84-4.86); L-lactate 43.38 mmol/litre (range 42.56-44.08).

Because of the high degree of reproducibility single incubations were carried out.

Experiment 1
First, the effect of a number of feedstuffs on pH and on lactate production was studied during long-lasting incubations. In this experiment 1-g amounts of the feedstuffs were incubated for 7 hours. Feedstuffs in this experiment were mainly ingredients which are normally used in commercial concentrate mixtures. In addition, some purified carbohydrates and some commercial concentrate mixtures were chosen as substrates.

Experiment 2: dosage level
Incubations were performed in part with the same feedstuffs as used in Exp. 1 but taken from other batches. Feedstuffs were chosen on the basis of the differences in pH and lactate concentration they caused in the preceding experiment. The occurrence of substantial differences in sugar, starch and protein content was another decisive factor in the choice of the feedstuffs. These feedstuffs, i.e. maize gluten feed, maize, citrus pulp, tapioca, beet pulp, coconut expeller, soya bean meal (solv.extr.) are used in subsequent experiments as well. In Exp. 2 different quantities of each feedstuff were incubated for 4 hours with 20 ml rumen fluid, namely 0.25 g, 0.5 g and 1.0 g of the feedstuffs (Exp. 2A; 7 feedstuffs) and 0.5 g, 1.0 g, 1.5 g, 2.0 g, 2.5 g and 3.0 g (Exp. 2B; 4 feedstuffs). The pH was measured every hour.

Experiment 3: particle size
The substrates used in most of the in vitro experiments reported here were always milled through a 0.2-mm screen. Since feedstuffs are usually less finely milled (approximately 2.0-mm screen) in practice, the effect of particle size was studied.

The 7 ingredients (from the same batch as those in Exp. 2) were incubated in 1.0-g amounts with 20 ml of the rumen fluid for 6 hours and the finely milled form compared with the unmilled samples. The original batch of maize meal had already been milled through a 2.0-mm screen.

Experiment 4: effect of pelleting
To investigate a possible effect of pelleting, samples of a commercial mixture –

taken before and after pelleting – were incubated for 6 hours. The unpelleted mixture (2.0-mm screen) (A) was compared with the pelleted mixture (B) and with the pellet milled afterwards (0.2-mm screen) (C).

The ingredient composition (g/kg) of the concentrate mixture used in Exp. 4 is presented in Table 1.

Experiment 5: variability between batches

To see whether differences could be observed between batches of a feedstuff in their effect on pH or on lactic acid concentration after incubation, samples were taken from several batches of a number of feedstuffs and subjected to 4-hour incubations. In this experiment 1.0 g of each feedstuff was incubated with 20 ml of rumen fluid. For each feedstuff pH was measured hourly, but within feedstuffs lactic acid was analysed only in the flasks showing the highest and the lowest pH after incubation.

Experiment 6: combinations of ingredients

An experiment was carried out with the 7 feedstuffs from the same batches as in Exp. 2 and 3 and with a number of combinations of these feedstuffs. With the combinations it was our aim to form 2 mixtures with equal protein content (crude protein about 160 g/kg). One of these mixtures contained feedstuffs (250 g/kg each of maize meal, tapioca, beet pulp and soya bean meal) conferred little danger of causing a high acidity. The other mixture contained as much as possible feedstuffs with a high risk of causing acidosis (333 g/kg each of citrus pulp, maize gluten feed and coconut meal). Also an intermediate mixture was composed, but here a lower protein content could not be avoided (crude protein about 110 g/kg). Molasses and minerals plus vitamins (10 mg calcium phosphate + 10 mg NaCl + 5 mg premix per incubation) were also added to the two mixtures. In all cases a total of 1.0 g of substrate was incubated with 20 ml of rumen fluid for 4 hours; only the minerals plus vitamins were added on top of the 1.0 g of substrate.

Table 1. Ingredient composition (g/kg) of the concentrate mixture used in Experiment 4.

Feedstuff	Content (g/kg)
Maize gluten feed meal	250
Citrus pulp	180
Soya bean meal (solv. extr.)	60
Rice feed meal	40
Palm kernel meal	100
Wheat meal	30
Grass meal	30
Wheat midlings	180
Molasses	80
Animal fat	10
Minerals + vitamins (premix)	40

Experiment 7: addition of some proteins and of Chromosorb
In the preceding experiment it was established that with combined feedstuffs – especially when tapioca was included – often more lactic acid was formed and the pH became lower than was expected. To get an understanding of the possible cause of this phenomenon the following experiment was carried out. To maize and to tapioca (same batches as in preceding experiments) casein, yeast extract or trypticase (a tryptic digest of casein), maize meal, soya bean meal, Chromosorb (an inert carrier material used in gas chromatography) or combinations of these products were added and incubated with 20 ml of rumen fluid for 4 hours (for quantities see Table 4).

Experiment 8: addition of some starches and sugars
The addition of casein, trypticase, yeast extract or Chromosorb to tapioca (Exp. 7) did not explain the differences found when tapioca was combined either with maize meal of with soya bean meal (Exp. 6). Therefore the next experiment was carried out. To maize meal, tapioca or soya bean meal (same batches as in preceding experiments) different kinds of starch (maize starch, potato starch or soluble starch) or of different sugars (glucose, maltose or cellobiose) were added and incubated for 4 hours with 20 ml of rumen fluid (for quantities see Table 5).

Results

Experiment 1
From Fig. 1A and 1B it appears that changes in pH and in the L-lactate concentration became noticeable after a lag time of about 2 hours. Upon further incubation, clear differences arose between feedstuffs, both in the decrease in pH as well as the increase in L-lactate is concerned. With sorghum hardly any L-lactate formation was observed, while a distinct accumulation of L-lactate was seen with wheat midlings coinciding with a sharp decrease in pH. After 4 hours of incubation with coconut meal L-lactate decreased (Fig. 1A), which coincided with a slower decrease in pH (Fig. 1B). Maize meal (data not presented) showed a similar pattern in the development of the L-lactate concentration as coconut meal but the pH continued to fall throughout the 6-hour incubation instead.

Maize gluten feed caused a lower pH and a higher L-lactate concentration at t = 1 than the other feedstuffs. This suggests that this batch of maize gluten feed contained some lactic acid.

The pH values measured at hourly intervals for 6 hours were added for each of the individual feedstuffs and the same was done for the L-lactate concentrations. This was done to obtain clear differences between feedstuffs. In Fig. 1C the relationship between the sum of pH values and the sum of the L-lactate concentrations is presented. Since the pH was 6.3 at the start of the experiments, the sum of the pH values should equal 37.8 in case no change was observed in pH during the 6-hour incubation period.

From Fig. 1C it appears that a decrease in the sum of the pH values coincided with an increase in the sum of the L-lactic acid concentrations. The feedstuffs

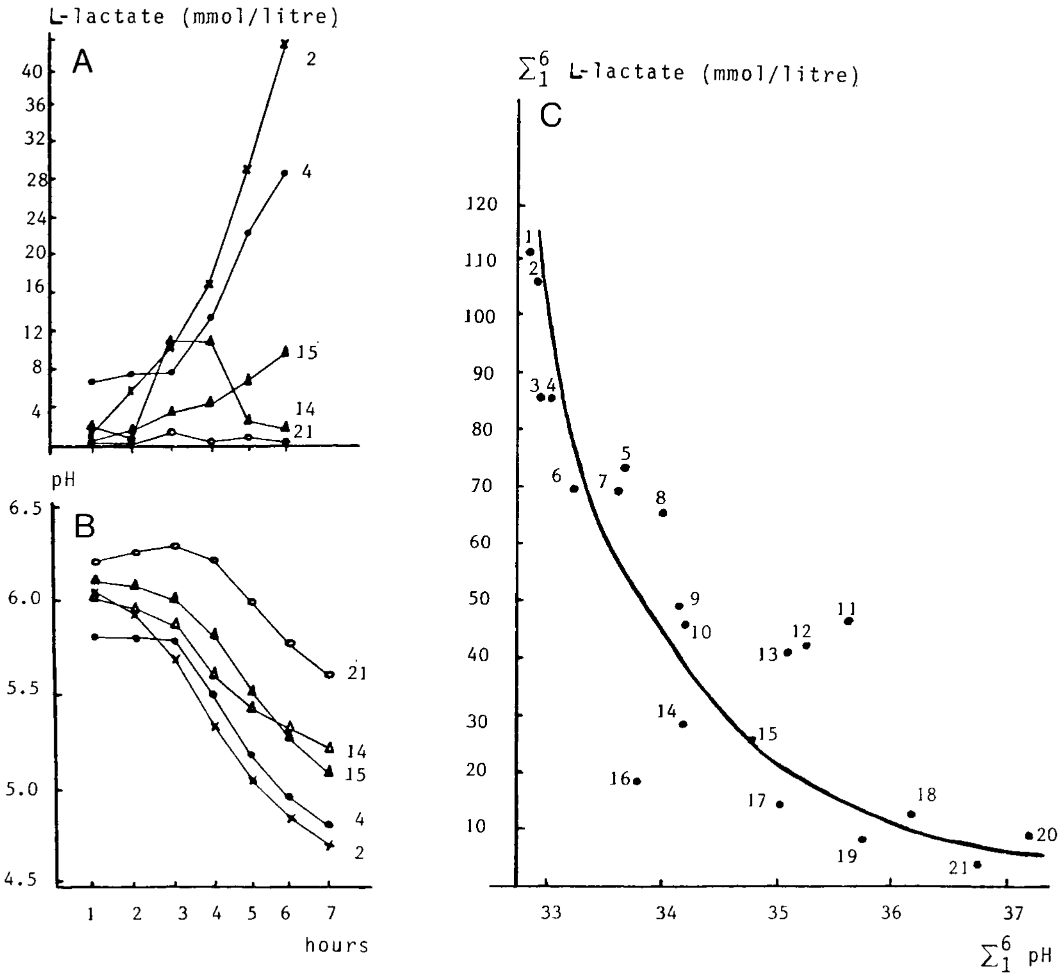

Fig. 1A (top left). L-lactate concentrations after incubation of 1 g of several feedstuffs in 20 ml of rumen fluid for 6 hours.
Fig. 1B (bottom left). pH values after incubation of 1 g of several feedstuffs in 20 ml of rumen fluid for 7 hours.
Fig. 1C (right). Relationship between summarized pH* and summarized L-lactate* concentration after incubation of several feedstuffs in 20 ml of rumen fluid for 6 hours.
1 = mixture 1978**; 2 = wheat midlings; 3 = beet pulp; 4 = maize gluten feed; 5 = molasses; 6 = citrus pulp; 7 = mixture 1979**; 8 = soya bean meal (solv. extr.); 9 = sucrose; 10 = rape-seed meal (solv. extr.); 11 = soluble starch; 12 = maize meal; 13 = pea meal; 14 = coconut meal (expeller); 15 = oat meal; 16 = hominy feed; 17 = linseed meal (expeller); 18 = horse bean meal; 19 = tapioca; 20 = maize starch; 21 = sorghum meal.
* Σ_1^6 = result after 1 h + result after 2 h + . . . + result after 6 h.
** For ingredient composition see Malestein et al., 1981.

soluble starch, maize and peas showed a strong increase as compared to the other products. On the other hand hominy feed showed a small increase in lactic acid concentration in comparison with the decrease in pH.

Experiment 2: dosage level

The pH values – measured at hourly intervals – after 4-hour incubations are presented in Fig. 2. As was to be expected, pH became lower when the dose of the substrate increased. On closer inspection there were some striking differences between the feedstuffs. As the quantity of the substrate (maize gluten feed and citrus pulp) increased from 0.25 g to 0.50 g, pH decreased strongly, but with a further increase in substrate quantity hardly any change in pH could be

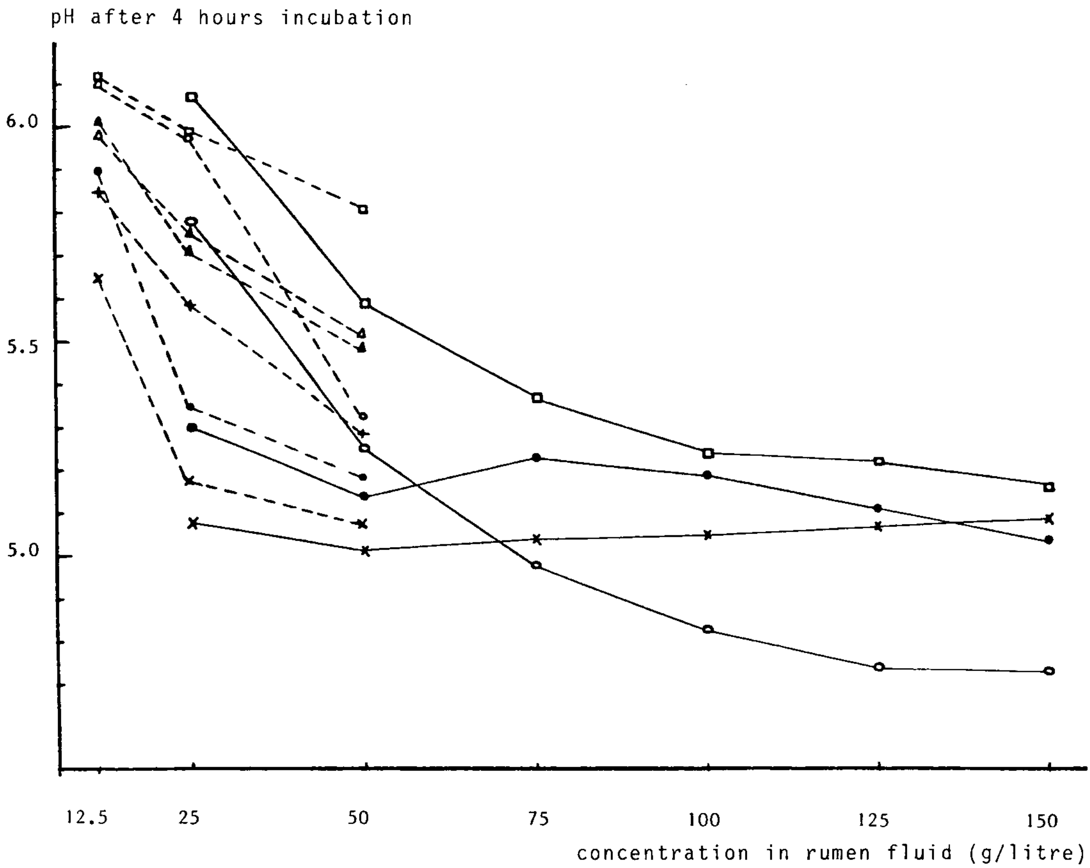

Fig. 2. pH values after 4 h of incubation of different amounts of concentrate ingredients with rumen fluid in vitro.
- - - - - Exp. 2A ——Exp. 2B.
□ tapioca; ○ maize meal; ▲ soya bean meal (solv. extr.); △ coconut meal (expeller); ● maize gluten feed; + beet pulp; × citrus pulp.

observed. With maize meal pH decreased strongly when the substrate was increased to 2.0 g or 2.5 g. When the feedstuffs were indexed according to the pH decrease there is a strong inversion in index with substrate levels from 0.25 g to 3.0 g per flask.

Experiment 3: particle size

Particle size of the feedstuffs hardly had an influence on the formation of lactic acid or on the development of the pH during the incubations in vitro, except with maize meal (Fig. 3). With smaller particle size of maize meal, L-lactate concentration increased coinciding with a lower pH.

In this experiment, at t = 1, L-lactate concentration with maize gluten feed was higher than with the other feedstuffs, as was found also in Exp. 1.

Experiment 4: effect of pelleting

Concentrates for dairy cattle usually are pelleted. In this process steam is used mostly, and during pelleting temperature increases strongly. This could result in

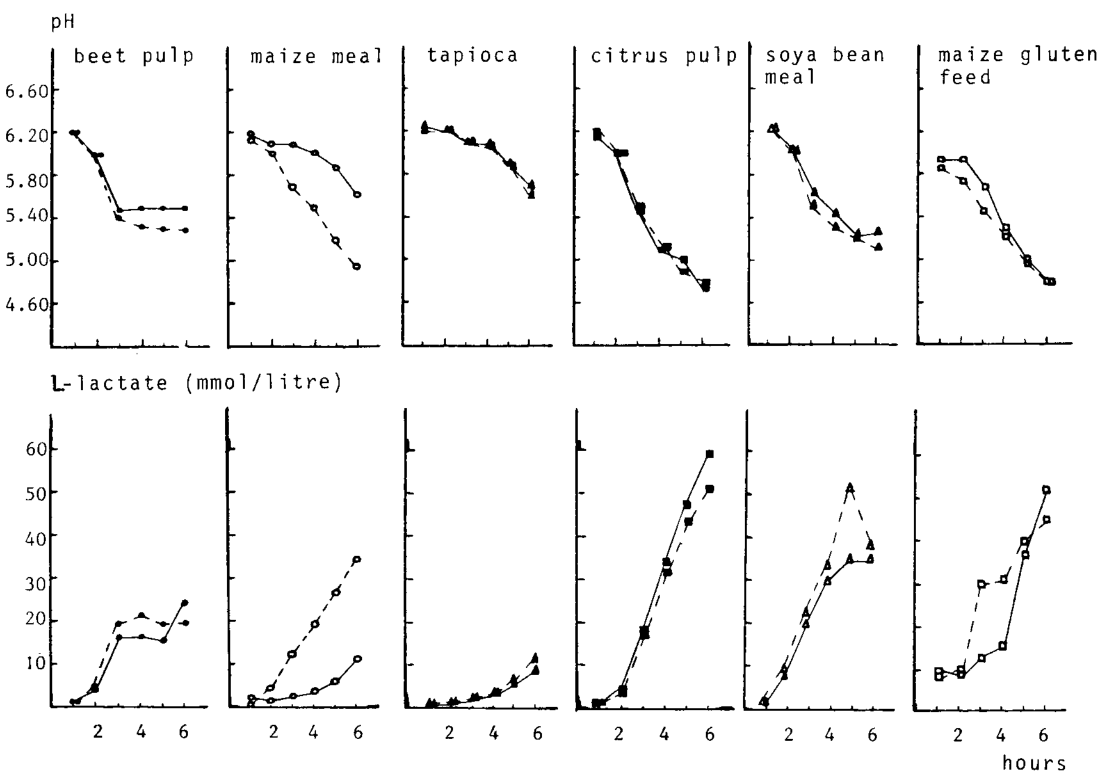

Fig. 3. Influence of particle size on pH and on L-lactate concentration after a 6 hours incubation.
——— as manufactured (maize was milled through a 2-mm screen).
- - - - milled through a 0.2-mm screen.

a certain toasting of the starch, which could result in a faster fermentation. After 6 hours pH decreased to 5.91, 5.89 and 5.85 respectively for A, B and C, and the L-lactate concentrations were 57, 62 and 66 mmol/litre respectively. From this it appeared that pelleting the mixture hardly had an influence on pH or on L-lactate concentrations during incubation in vitro. Renewed milling of the pelleted concentrate mixture had no appreciable influence, which agreed with the results of Experiment 3.

Experiment 5: variability between batches

Table 2 shows that sometimes (e.g. beet pulp and soya bean meal) considerable differences appeared in pH or L-lactate concentration or both. The greatest difference was observed for the pH in the incubations with maize meal. High concentrations of lactic acid were associated with low pH values.

With maize meal the decrease in pH as compared with the increase in lactic acid concentration was greater than, for example, with soya bean meal. It is not likely that the higher protein content of soya bean meal is responsible for this difference, since with hominy feed a similar effect was seen. Production of different amounts of volatile fatty acids is a more reliable explanation for the different effects.

This experiment indicated once more that maize gluten feed contained some lactic acid. After the start of the incubation pH was lower and L-lactate concentration was higher with maize gluten feed than with the other feedstuffs and remained unchanged during the first 2 hours.

Experiment 6: combinations of ingredients

The results of pH measurements and analyses of the L- and D-lactic acid concentration after 4 hours of incubation with the single feedstuffs and with some of

Table 2. pH and L-lactate concentration (mmol/litre) after 4 hours of incubation with a number of feedstuffs from different batches.

Feedstuff	n	pH			L-lactate (mmol/litre)	
		mean	highest	lowest	lowest	highest
Citrus pulp	5	5.06	5.11	4.93	21.51	22.54
Beet pulp	6	5.55	5.62	5.32	5.18	9.02
Maize meal	6	5.42	5.74	5.29	3.61	10.53
Soya bean meal (solv. extr.)	8	5.41	5.65	5.33	9.91	30.40
Maize gluten feed	5	5.15	5.24	5.02	22.81	26.91
Tapioca	5	5.47	5.54	5.42	8.37	12.46
Wheat midlings	5	5.02	5.08	4.95	28.58	30.66
Coconut meal (expeller)	5	5.41	5.56	5.30	15.77	26.15
Rape-seed meal (solv. extr.)	4	5.31	5.42	5.24	17.29	25.76
Hominy feed	6	5.12	5.18	5.05	25.51	28.60

Table 3. pH and L- en D-lactate concentration (mmol/litre) after 4 hours of incubation with single or combined feedstuffs.

Feedstuffs	pH		L-lactate (mmol/litre)		D-lactate (mmol/litre)		D-lactate (ml/litre of total lactate)
	measured	calculated	analysed	calculated	analysed	calculated	
1. maize meal	5.14		22.03		4.74		177
2. tapioca	5.73		8.25		1.60		162
3. beet pulp	5.07		21.64		4.35		167
4. soya bean meal (solv. extr.)	5.07		32.85		5.55		145
5. citrus pulp	4.98		32.59		3.59		97
6. maize gluten feed	4.99		22.54		11.18		332
7. coconut meal (expeller)	5.38		15.85		4.74		230
8. molasses	5.24		18.16		5.53		233
1 + 2*	5.01	5.43	31.69	15.14	4.38	3.17	121
1 + 2 + 4*	4.89	5.32	35.17	21.04	5.96	3.96	145
1 + 2 + 3 + 4	4.90	5.25	28.86	28.26	5.37	4.06	157
3 + 4	5.07	5.07	18.29	27.25	5.44	4.95	229
1 + 2 + 3 + 4 + 8	4.82	5.25	27.70	20.59	6.27	4.35	185
5 + 7	5.11	5.18	22.80	24.22	3.83	4.17	144
5 + 6 + 7	4.87	5.12	34.01	23.66	7.19	6.50	175
5 + 6 + 7 + 8	4.82	5.15	37.62	22.29	6.58	6.26	149
3 + 5	4.99	5.03	27.05	27.12	3.84	3.97	124
1 + 3 + 5 + 6	4.83	5.05	34.40	24.70	6.20	5.97	153

* 1 + 2 = maize meal + tapioca; 1 + 2 + 4 = maize meal + tapioca + soya bean meal, etc.

the combinations are presented in Table 3. In addition to the measured values of pH and lactic acid concentrations calculated values of pH and of lactic acid concentrations are given. These were calculated on the assumption that effects of single feedstuffs were simply additive. Mathematical means of the final pH and of lactic acid concentrations from incubations with the single feedstuffs are presented.

From Table 3 it can be seen that with combined feedstuffs the measured pH was generally lower and the measured L- and D-lactate concentration higher than the calculated values. In some cases such as 3 + 4 (soya bean meal + beet pulp), 5 + 7 and 3 + 5 there was almost no difference between measured and calculated pH, while in others such as 1 + 2, 1 + 2 + 4 and 1 + 2 + 3 + 4 + 8 there were large differences. Differences between measured and calculated pH values and L-lactate concentrations were large when tapioca and to a lesser extent when maize gluten feed formed part of the combination. With 3 + 4 a considerable difference between measured and calculated L-lactate concentrations existed, but no difference between measured and calculated pH values was found with this mixture.

In general, D-lactic acid concentrations were low and amounted to 100-330 ml/litre of total lactic acid.

Adding minerals and vitamins to 1 + 2 + 3 + 4 + 8 or to 5 + 6 + 7 + 8 had no influence on pH but tended to cause somewhat higher lactic acid concentrations.

Experiment 7: addition of some proteins and of Chromosorb
Values for pH and L-lactate concentrations after 4 hours of incubation are given in Table 4. Incubation of 0.5 g maize meal or 0.5 g tapioca showed the expected differences (according to Exp. 2) and the addition of 10 mg of casein to maize meal or to tapioca in both cases resulted in a lower pH and in a doubling of the (small) L-lactate concentration. Addition of 10 mg of trypticase or of 10 mg of yeast extract to maize meal had some effect, but addition of these substances to tapioca had no effect. The same differences in effect were seen when a combination of yeast extract and trypticase was added to maize meal and to tapioca.

Addition of 0.5 g of maize meal or of 0.5 g of soya bean meal to maize meal or to tapioca again showed the effect as seen in Exp. 6. Addition of Chromosorb or of Chromosorb together with yeast extract and trypticase to maize meal had some effect, but none when added to tapioca.

Table 4. Effect on pH and on L-lactate concentration (mmol/litre) of the addition of several substances to maize meal or to tapioca (4 hours of incubation).

Feedstuff + addition	L-lactate	pH
0.5 g maize meal	1.51	6.17
+ 10 mg casein	3.67	6.04
+ 10 mg yeast extract	3.45	6.03
+ 10 mg trypticase	4.46	6.06
+ 5 mg yeast extract + 5 mg trypticase	5.89	5.99
+ 0.5 g maize meal	20.76	5.31
+ 0.5 g maize meal	18.83	5.33
+ 0.5 g soya bean meal (solv. extr.)	ca. 30	5.11
+ 0.5 g Chromosorb	4.60	6.03
+ 0.5 g Chromosorb + 5 mg yeast extract + 5 mg trypticase	8.48	5.93
0.5 g tapioca	0.63	6.34
+ 10 mg casein	1.19	6.29
+ 10 mg yeast extract	0.57	6.28
+ 10 mg trypticase	0.32	6.28
+ 5 mg yeast extract + 5 mg trypticase	0.46	6.23
+ 0.5 g maize meal	22.14	5.17
+ 0.5 g tapioca	6.76	5.99
+ 0.5 g soya bean meal (solv. extr.)	ca. 30	5.04
+ 0.5 g Chromosorb	0.66	6.23
+ 0.5 g Chromosorb + 5 mg yeast extract + 5 mg trypticase	0.70	6.26

Experiment 8: addition of some starches and sugars

From Table 5 it appears that incubation of 0.5 g of maize starch, of potato starch or of soluble starch hardly caused any formation of L-lactic acid while the pH remained unchanged. The incubation of 0.5 g of glucose, of maltose or of cellobiose did induce formation of L-lactic acid, which agreed with the decrease in pH. The incubation of 0.5 g of maize meal, tapioca or of soya bean meal showed the expected results (based on results of Exp. 2 and 7), but differences between these feedstuffs were rather large both when 0.5 g and when 1.0 g amounts were incubated.

Addition of 0.5 g of maize starch to 0.5 g of maize meal stimulated L-lactic acid formation and depressed the pH. When 0.5 g of maize starch was added to 0.5 g of tapioca or to 0.5 g of soya bean meal, pH remained higher and L-lactic acid concentrations remained lower than without the addition of maize starch. However, in this case the total concentration was 1.0 g, and the effect of the addition of 0.5 g of maize starch and of 0.5 g of potato starch therefore must be compared with the results of the incubation of 1.0 g of maize meal, of tapioca or of soya bean meal. If that is done the effect of the addition on L-lactic acid formation appeared to be negative and the effect on pH was strongly positive.

Addition of 0.5 g of soluble starch to 0.5 g of maize meal depressed the production of lactic acid, while the addition of this substance to 0.5 g of tapioca stimulated lactic acid production. Soluble starch addition (0.5 g) to 0.5 g of soya bean meal had no effect. Addition of 0.5 g of soluble starch to 0.5 g amounts of maize meal, tapioca or soya bean meal respectively always resulted in a lower

Table 5. Effect on pH and on L-lactate concentration of the addition of different starches or sugars to maize meal, tapioca or soya bean meal (4 hours of incubation).

Feedstuff + addition	L-lactate (mmol/litre)	pH	Feedstuffs + addition	L-lactate (mmol/litre)	pH
0.5 g maize starch	0.01	6.50	+ 0.5 g potato starch	0.49	6.41
0.5 g potato starch	0.03	6.51	+ 0.5 g soluble starch	6.79	6.02
0.5 g soluble starch	1.01	6.34	*0.5 g tapioca (continued)*		
0.5 g glucose	11.30	5.61	+ 0.5 g glucose	40.04	5.02
0.5 g maltose	4.56	6.12	+ 0.5 g maltose	20.67	5.35
0.5 g cellobiose	9.47	5.81	+ 0.5 g cellobiose	29.25	5.25
0.5 g maize meal	10.56	5.69	+ 0.5 g tapioca	3.22	6.11
+ 0.5 g maize starch	14.99	5.39	*0.5 g soya bean meal*	12.04	5.89
+ 0.5 g potato starch	12.33	5.78	+ 0.5 maize starch	4.82	5.98
+ 0.5 g soluble starch	18.42	4.90	+ 0.5 potato starch	7.06	6.01
+ 0.5 g glucose	32.98	4.66	+ 0.5 soluble starch	49.53	5.10
+ 0.5 g maltose	32.08	4.70	+ 0.5 g glucose	67.47	4.72
+ 0.5 g cellobiose	34.14	4.71	+ 0.5 g maltose	69.81	4.78
+ 0.5 g tapioca	36.71	4.86	+ 0.5 g cellobiose	60.16	4.74
+ 0.5 g maize meal	28.34	5.02	+ 0.5 g tapioca	63.18	4.88
0.5 g tapioca	2.56	6.12	+ 0.5 g soya bean meal	51.74	5.17
+ 0.5 g maize starch	0.40	6.32			

pH as found after incubation of 1.0 g amounts of these substrates respectively. Addition of 0.5 g of glucose, of maltose or of cellobiose to 0.5 g of maize meal, tapioca or soya bean meal respectively stimulated lactic acid formation and declined the pH as compared with the lactic acid concentration and the pH found after incubation of 1.0 g of maize meal, tapioca or soya bean meal respectively. Addition of 0.5 g of tapioca to 0.5 g of maize meal or of soya bean meal proved to have an effect equal to that of addition of sugars to maize meal or to soya bean meal. This indicates that tapioca, when combined with other products, reacts more like a sugar and behaves less like a starch product.

Discussion

Single feedstuffs
In our in vitro experiments highly different lactic acid concentrations and pH values were found after 4-hour incubations of single feedstuffs with rumen fluid from hay-fed cows. From ninefold incubations with tapioca and tenfold incubations with citrus pulp (one batch each) in one experiment reproducibility proved to be very high. However, when in repetition experiments feedstuffs of the same batches were incubated, level of pH and of L-lactate concentrations differed. Moreover, when feedstuffs were indexed according to final pH or lactic acid concentration reached after 4-hour incubations, sometimes the index slightly changed in repetition experiments. Possibly a varying microbial composition of the rumen fluid was responsible for this effect. Index of feedstuffs according to the pH reached after 4 hours of incubation changed strongly when the dose of the substrate increased from 0.25 to 2.0 g per 20 ml of rumen fluid. This shows the dependence of the acidotic index from the substrate quantity.

Abe & Kandatsu (1978) incubated several feedstuffs for 3 or 8 hours in vitro with rumen microbes and found a lower pH when maize starch was incubated than when wheat bran, maize meal of soya bean meal were incubated. When we index the feedstuffs used in the experiments of Abe & Kandatsu (1978) according to final pH, the index obtained does not agree with the index found in our experiments. Nocek et al. (1979) found with the nylon bag technique that wheat bran was degraded faster than, for example, maize meal or soya bean meal. It is likely that with a faster rate of degradation of the feedstuffs, the pH in the rumen fluid will become lower. In the experiments of Nocek et al (1979) ranking of feedstuffs according to the rate of degradation agreed rather good with the acidotic index in our experiments.

In our experiments an effect of particle size on the decrease of pH and on L-lactate concentration after 4 hours of incubation was found when maize meal was used, but not with the other feedstuffs studied. When feeding maize to steers Galyean et al. (1979) found that particle size influenced the rate of rumen digestion. In their experiments fineness of maize was less than that in our experiments. An influence of particle size on dry matter disappearance in vitro has been described by Berger et al. (1981) and Walker et al. (1973) for other cereal grains.

Combined feedstuffs
When feedstuffs were combined and incubated for 4 hours usually the final pH was lower and the L-lactate concentration was higher than when the feedstuffs were incubated separately. For this some explanations are possible. First, when substrate has a more variable composition more species of micro-organisms can ferment part of it and as a consequence more volatile fatty acids and/or lactic acid will be formed, resulting in a lower pH.

Secondly, an influence of the nitrogen source on lactic acid formation is possible (Counotte, 1981). In our experiments when casein or trypticase were added to tapioca almost no effect on L-lactate production or on pH decline could be observed, but such effects were marked when these N-containing substances were added to maize meal.

Lewis & McDonald (1958) carried out a series of experiments by placing different amounts of feedstuffs in the rumen of sheep, such as 100 g of casein or 250-g amounts of sucrose, glucose and starch separately or 100 g of casein combined with each of the 250-g amounts of these carbohydrates. When 100 g of casein were added to 250 g of sucrose, rumen lactic acid concentration was increased very strongly. But addition of 100 g of casein to 250 g of glucose or starch had hardly any positive effect on lactic acid concentration. These experiments of Lewis & McDonald (1958) therefore showed differences in results when a N source was added to different carbohydrates comparable to our findings (Exp. 7).

Thirdly, it is possible that bacteria attack substrate more easily when this substrate has a larger volume. Possibly the effect of addition of Chromosorb to maize meal could be an indication in this direction and can be compared with that of enlargement of the surface (as with milling).

De Visser (1980) studied the effect of varying percentages of starch and sugars on rumen fermentation by feeding concentrates with 200-500 g/kg of starch and sugars to dairy cows. The content of starch and sugars was raised mainly by increasing the proportion of tapioca. When the concentrates high in starch and sugars were fed, lactic acid concentration in rumen fluid was increased, while dry matter intake and milk production were decreased as compared with the situation when concentrates low in starch and sugars were fed. In our experiments the greatest effect on pH and on lactate concentration was found when tapioca was added to soya bean meal, while almost no lactic acid was formed when potato starch or maize starch were added to soya bean meal. Based on these results the effect on rumen fermentation as stated by de Visser (1980) might be relevant only with the specific ingredient composition used in his experiments.

Our experiments clearly show that the risk of lactic acid acidosis by concentrate mixtures cannot be predicted from in vitro incubations with single concentrate ingredients. It is also demonstrated that in studies on carbohydrate fermentation in the rumen it is necessary to define the sources of starch or sugar used.

Conclusions

1. After at least 4 hours of incubation in vitro of different feedstuffs with rumen fluid large differences in pH and in L-lactate concentration could be observed.
2. Increasing the concentration of the substrates clearly influenced the acidotic index of the feedstuffs.
3. Except with maize meal, hardly an effect of particle size on pH and on L-lactate concentration could be observed.
4. Pelleting of a concentrate mixture in itself had hardly an effect on profiles of pH and of L-lactate concentration.
5. Considerable differences in effect on pH and on L-lactate concentration were observed between different batches of the same feedstuffs especially with maize meal.
6. When incubations were carried out with mixtures of concentrate ingredients, pH and L-lactate concentrations differed from values calculated from results obtained with the single ingredients.

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