EFFECT OF SUPPLEMENTARY UREA, GLUCOSE AND MINERALS ON THE IN VITRO DEGRADATION OF LOW QUALITY FEEDS

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Summary

Increasing levels of ammonia-N in the rumen fluid used for in vitro incubation were achieved by supplementation of the ration of the donor cows with urea and by addition of urea either with or without glucose to the rumen fluid after collection. The ration of the donor animals consisted of wheat straw (80%) and maize silage (20%). During the second half of the experiment the basal ration was supplemented with a mineral mixture. Wheat straw, Guinea grass and two rice straw varieties were incubated with the various kinds of rumen fluid. Parameters studied were: solubility, apparent organic matter disappearance after 48 hours of incubation (OMD_{48}), rate of organic matter degradation from 0 to 24 hours of incubation (k_1) and from 24 to 96 hours (k_2). The concentration of ammonia-N in the rumen fluid at which 95% of the maximal OMD_{48} and k_1 were reached (88.2 and 100.0 mg/l) were independent of the feed. With regard to the k_2 the required ammonia-N concentration to reach 95% of the maximal k_2 differed per feed. Mineral supplementation increased the OMD_{48} and k_1, but not the solubility and k_2. Glucose addition in combination with urea had no beneficial effect compared to urea supplementation alone.

(**Key Words:** In Vitro Degradation, Low Quality Feeds, Urea Supplementation, Mineral Supplementation, Glucose Supplementation)

Introduction

Rumen microbes utilize ammonia – nitrogen (AN) for synthesis of microbial protein, but also for processes involved in degradation of the feed. Optimal AN concentration for maximal microbial growth is in the range of 20-50 mg/l (Satter and Slyter, 1974). Others mention much lower requirements for optimal growth rates of pure cultures of rumen microbes (Hespell and Bryant, 1979; Schaeffer et al., 1980) varying from 1.4 to 14 mg AN per liter. For optimal degradation of the feed higher AN concentrations are required (Mehrez et al., 1977; Erdman et al., 1986). Erdman et al. (1986) reported AN requirements for optimal degradation ranging from 40 to 250 mg/l dependent on the potential digestibility of the feed. Supplementation with urea of rations low in N may increase the intake and the digestibility of the dry matter, but in some cases the effect is very small and not significant (Campling et al., 1962; Orskov and Grubb, 1978; Kellaway and Leipholz, 1983; Dias-da-Silva and Sundstol, 1986; Schiere and Wieringa, 1988). The effect of urea supplementation on intake and digestibility could be dependent on the potential digestibility of the feed as suggested by Orskov and Grubb (1978).

The objectives of the presented experiments were:

1. to study the effect of the AN level of the substrate on the in vitro degradation of low quality feeds.
2. to study the effect of additional minerals and easily available carbohydrates on the in vitro degradation of low quality feeds.

Materials and Methods

Animals, basal diet and experimental design

Two Dutch Friesian non lactating cows cannulated with a large, 10 cm inside diameter rumen cannula (Bar Diamond Inc. Parma, ID, USA) were used. The cows were individually tethered in

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tie stalls at the Institute for Animal Feeding and Nutrition Research at Lelystad, The Netherlands and had *ad libitum* access to water. The basal diet consisted of chopped (2 cm) wheat straw (80% on dry matter basis) and maize silage (20% on dry matter basis). The composition of these feeds is given in table 1. At 5.30 a.m. 40% of the ration was offered, the rest at 17.00 p.m.. Previous to the experiment the animals were fed on the basal ration for about 4 months. It was assumed, that by that time full adaptation to the ration was achieved. The experiment consisted of 4 periods. During period 1 one of the cows received the basal ration, while the other received the same but

TABLE 1. COMPOSITION OF THE BASAL RATION

Feed	Wheat straw	Maize silage	Total ration[1]
DM (%)	95.3	23.7	59.5
OM (% in DM)	86.5	93.2	87.8
CP (% in DM)	2.4	9.6	3.8
OMD (%)[2]	40.5	71.0	46.6

[1] 80% wheat straw, 20% maize silage.
[2] Organic matter digestibility measured by an in vitro method (two stage Tilley and Terry) with calibration line for relation in vitro - in vivo for comparable feeds.

supplemented with urea (2% of DM). In period 2 the rations of the cows were changed. During period 3 one of the cows received the basal ration supplemented with 236 grams of a commercial mineral mixture containing Ca, P, Mg, Na, Cl and S, micro-elements, vitamine A and vitamine D, while the other animal received the basal ration supplemented with urea (2% of DM) and 236 grams of the mineral mixture and 35 grams of sodium-sulphate to get a N/S – ratio of 10/1. In period 4 the diets of the animals were changed. Each period had a duration of 42 days of which 24 days were adaptation period. Rumen fluid (2 liters of each animal) was collected at day 25 and 32 of each period at 7 a.m., 1.5 hours after feeding. The rumen fluid was collected in preheated thermosflasks (39 °C) and transported to Wageningen, where it arrived about 1 hour after collection. The average temperature of the rumen fluid at arrival in Wageningen was 34°C.

Four test feeds, wheat straw, guinea grass and two rice straw varieties were incubated with the rumen fluid. The composition of these feeds is given in table 2. All feeds except the wheat straw were collected in Sri Lanka. The wheat straw was the same as fed in the basal ration of the donor animals. All test feeds had a high cell wall and a low N content.

TABLE 2. COMPOSITION OF THE TEST FEEDS

Feed	Wheat straw	Guinea[1] grass	Rice straw[1] BG297-2	Rice straw[1] BG298-2
DM (%)	95.3	94.7	95.0	94.1
OM (% of DM)	86.5	90.5	85.6	83.6
CP (% of DM)	2.4	4.4	4.2	3.6
NDF (% of DM)	78.3	76.3	71.4	71.0
ADF (% of DM)	49.4	52.0	43.5	43.0
Lignin (% of DM)	5.5	7.6	3.4	3.5
Digestibility of OM (%)[2]	40.5	42.5	55.7	48.7

[1] Feeds from Sri Lanka. The Guinea grass was harvested at a mature stage (about 10 weeks regrowth).
[2] Measured by in vitro method (two stage Tilley and Terry) with calibration line for relation in vitro - in vivo for comparable feeds.

The feeds were ground to pass a 1 mm sieve and 500 mg of air dry material was weighed into 150 ml bottles, that were closed with a rubber stopper with a valve to let fermentation gasses out and to prevent influx of oxygen. The bottles with feeds were stored at 39 °C till the moment of incubation. The rumen fluid of each cow was strained through four layers of cheese cloth after which it was added to a carbon dioxide saturated sodium bicarbonate buffer (Tilley and Terry, 1963) at a temperature of 39°C.

The concentration of rumen fluid in the buffer solution was 20 %, and 50 ml of the rumen fluid buffer solution was added to the feed samples in the bottles. The bottles were put in an incubator at 39°C and shaken twice daily.

To study the kinetics of degradation, incubation was terminated after 0.5, 24, 48 and 96 hours by filtering over glass filter crucibles (Duran no 2; poresize 40-100 μm). The residues were washed

with warm water. The incubation period of 0.5 hours was applied to study the solubility of the test feeds.

The organic matter residue after incubation of t hours (OMR_t) was calculated from the weight of the crucible plus contents after drying minus the weight of the crucible plus contents after ashing and was corrected for a blank. The OMR_t was expressed as percentage of the organic matter quantity in the sample before incubation. The apparent organic matter disappearance at time t (OMD_t) is equal to (100-OMR_t).

The following supplements were added to the test feeds in the bottles just before addition of the rumen fluid/buffer solution:

- none
- 7.5 mg urea (Merck 8486)
- 7.5 mg urea and 35.3 mg glucose (Merck 8342).

Glucose was added to see whether easily available energy would affect the effect of urea supplementation.

Within each of the four periods there were two runs of in vitro incubation. In the first run no supplement and the urea supplementation were examined and in the second run no supplement and the supplementation of urea plus glucose.

The AN content of the substrate, when no urea supplement was added to the bottles was equal to the concentration of AN in the rumen fluid divided by 5, since there was 20% of rumen fluid in the substrate. The amount of urea added to the substrate for the in vitro incubation increased the N-content of the substrate with 70 mg/l. All substrates in which the test feeds were incubated are given in table 3. Within each substrate existed a large variation in the AN content. Therefore the AN content was treated as a continuous variable for statistical analysis.

TABLE 3. COMPOSITION OF SUBSTRATES IN WHICH THE TESTFEEDS WERE INCUBATED (MEANS AND STANDARD DEVIATION)

Treatments				
Cow diet		In vitro supplement		AN
Urea	Minerals	Urea	Glucose	Level (mg/l)
–	–	–	–	12.8 (4.7)
+	–	–	–	47.9[1] (7.5)
–	+	–	–	9.3 (2.5)
+	+	–	–	41.9[1] (14.2)
		+	+ and	82.8[2] (4.7)
+	–	+	+ and –	117.9[2] (7.5)
–	+	+	+ and –	79.3[2] (2.5)
+	+	+	+ and –	111.9[2] (14.2)

[1] AN level due to in vivo supplementation of urea.

[2] AN level due to in vivo and in vitro urea supplementation. Value is equal to the level due to in vivo supplementation plus 70 mg/l due to in vitro supplementation of urea.

Dry matter, ash and Kjeldahl-N were determined by standard methods. Cell wall analysis was done by the methods of Goering and Van Soest (1970). Samples for analysis of the composition of the rumen fluid were taken immediately after arrival in the laboratory. Measured were pH, total N content by standard Kjeldahl procedure, AN by distillation and titration (i.e. the last part of the Kjeldahl procedure) and volatile fatty acids by gas chromatography.

The rate of degradation of the organic matter, defined as the percentage of total organic matter apparently degraded per hour was calculated for the time intervals from 0 to 24 hours (k_1) and from 24 to 96 hours (k_2) by the formulas:

$k_1 = (OMD_{24}\text{-}OMD_{0.5})/24$

$k_2 = (OMD_{96}\text{-}OMD_{24})/72$

Analysis of variance and covariance was done with SAS computer programmes (SAS, 1985).

Full models used for statistical analysis were:

1. $Y_{ijk} = \text{mean} + M_i + U_j + M_i{*}U_j + e_{ijk}$ for analysis of the effect of in vivo mineral supplementation (M) and urea supplementation (U) on the various parameters concerning the composition of the rumen fluid (Y). The number of data in each analysis was 16.
2. $Y_{ijklmn} = \text{mean} + M_i + G_j + F_k + C_l + b * AN_m + c * AN_m{*}AN_m + \text{two-way interactions} + e_{ijklmn}$ for analysis of the effect of glucose as an additional supplement to urea supplementation in vitro. M is the mineral effect, G the glucose effect, F the feed effect, C the cow effect and AN the AN level (mg/l) of the substrate and Y is either k_1, k_2 or OMD_{48}. Only the results from incubations with in vitro supplementation of urea and urea plus glucose were analysed. The number of data was 56. Eight values were missing.
3. $Y_{ijklm} = \text{mean} + M_i + F_j + C_k + b * AN_l +$

c * AN_j * AN_l + two-way interactions + e_{ijklm} for analysis of the effect of mineral supplementation (M), the feed effect (F), the cow effect (C) and the AN level of the substrate (AN mg/l) on the degradation parameters (Y). The number of data for this analysis was 112. Sixteen values were missing.

The significant factors, co-variables and interactions were put in the models by which least square means (lsmeans) and regressions were calculated. No significant ($p > 0.05$) difference existed between period 1 and period 2 nor between period 3 and period 4 in the in vitro degradation. For that reason it was assumed, that there was also no difference between the first two and the last two periods. The period effect was therefore not taken up in the statistical models.

Results

Composition of the rumen fluid

The composition of the rumen fluid one hour after feeding as affected by the rations of the donor animals is given in table 4. The pH of the rumen fluid 1 hour after feeding was significantly reduced by mineral addition, but was not affected by the urea supplementation. The VFA-content of the rumen fluid increased due to urea and mineral supplementation. The C2/C3-ratio was lowest, when no supplement was added. There was no difference between the C2/C3 ratios, when the donor animals received urea, minerals or both supplements. The AN content and non ammonia-N content (NAN, which is equal to total N minus AN) 1 hour after feeding were significantly increased when urea was added to the ration of the donor animals. Data concerning intake and rumen parameters of the donor animals will be published elsewhere.

TABLE 4. COMPOSITION OF THE RUMEN FLUID 1 HOUR AFTER FEEDING AS AFFECTED BY SUPPLEMENTATION OF THE DONOR ANIMALS

Ration	Without urea		With urea		Significances		
	−M[1]	+M[2]	−M	+M	Urea	M	Urea*M
pH	7.10	6.90	7.22	6.88	ns	*	ns
VFA (mmol/l)	68.9	76.8	73.8	80.5	*	*	ns
C2/C3-ratio	2.92	3.74	3.51	3.54	ns	*	*
NH3-N (mg/l)	64.0	43.0	239.3	210.8	***	ns	ns
NAN (mg/l)	151.8	169.4	192.4	233.8	*	ns	ns

* $P < 0.05$, *** $P < 0.001$
[1] no mineral supplementation.
[2] mineral supplementation.

TABLE 5. LEAST SQUARE MEANS OF DEGRADATION PARAMETERS FOR IN VITRO INCUBATION WITH ADDITION OF UREA AND UREA PLUS GLUCOSE TO THE SUBSTRATE (BETWEEN BRACKETS SEM)

In vitro supplement	Urea	Urea plus glucose
OMD_{48}[1] (%)	40.19 (0.69)	39.13 (0.70)
k_1[1] (%/hr)	0.81 (0.02)	0.77 (0.02)
k_2[2] (%/hr)	0.33 (0.01)	0.32 (0.01)

[1] means corrected for mineral effect, feed effect and AN level.
[2] means corrected for feed effect.

Effect of additional glucose to urea supplementation on degradation parameters

Significant effects in the full model by which the effect of additional glucose to urea supplementation in vitro was analysed were: the mineral effect, the feed effect and the AN level (linear) for OMD_{48} and k_1 and only the feed effect for k_2. The glucose effect was not significant as is illustrated in table 5 in which the lsmeans and the standard error of the lsmeans (SEM) of the degra-

dation parameters for urea plus glucose addition and for urea supplementation alone are given. For further analysis of the data no distinction was made between the two treatments.

The effect of AN level and mineral level of the substrate on in vitro degradation parameters

Significant effects in the statistical models by which the effect of AN level and minerals on the in vitro degradation parameters were analysed were:

for the $OMD_{0.5}$: Feed
for the k_1: Mineral, Feed, AN, AN*AN
for the k_2: Feed, Feed*AN, Feed*AN*AN
for the OMD_{48}: Mineral, Feed, AN, AN*AN.

The OMD_{48} and the k_1 were higher when the rumen fluid in which the test feeds were incubated was derived from donor animals, that received a mineral supplement (see table 6). The k_2 and the $OMD_{0.5}$ were not affected by this mineral supplementation. The interaction between minerals and the AN level was not significant, indicating, that the positive effect of mineral supplementation on OMD_{48} and k_1 was equal on all AN levels.

A feed effect was present in all degradation parameters. Guinea grass had the highest solubility (=$OMD_{0.5}$). The rice straw varieties were intermediate and did not differ from each other. The wheat straw had the lowest solubility (see table 7).

TABLE 6 LEAST SQUARE MEANS OF DEGRADATION PARAMETERS OF TEST FEEDS INCUBATED WITH RUMEN FLUID FROM ANIMALS ON A RATION WITH OR WITHOUT SUPPLEMENTARY MINERALS (BETWEEN BRACKETS SEM)

In vivo supplement	$OMD_{0.5}$[1] (%)	k_1[2] (%/hr)	k_2[2] (%/hr)	OMD_{48}[2] (%)
No minerals	7.01	0.551^{a}	0.284	30.18^{a}
	(0.26)	(0.015)	(0.009)	(0.47)
Minerals	7.59	0.674^{b}	0.282	34.81^{b}
	(0.28)	(0.016)	(0.011)	(0.49)

Different superscripts per column indicate significant differences ($p < 0.05$).
[1] means corrected for Feed.
[2] means corrected for Feed and AN level.

The difference between feeds with regard to k_1 were small. Guinea grass differed significantly from the rice straw varieties. The OMD_{48} of the wheat straw was lower than of the other feeds. All feeds differed from each other with regard to the k_2. Rice straw BG297-2 had the highest rate of degradation in the period from 24 to 96 hours after start of incubation and guinea grass was least degraded during this period. The k_2 was lower than the k_1 and was 43.7%, 32.9%, 55.1% and 51.2% of the k_1 for wheat straw, guinea grass, rice straw BG297-2 and rice straw BG298-2 respectively. The relation between OMD_{48} or k_1

TABLE 7. LEAST SQUARE MEANS FOR DEGRADATION PARAMETERS OF THE FOUR TESTFEEDS (BETWEEN BRACKETS SEM)

Feed	$OMD_{0.5}$[1] (%)	K_1[2] (%/hr)	K_2[3] (%/hr)	OMD_{48}[2] (%)
Wheat straw	3.32^{a}	0.606^{bc}	0.265^{b}	27.44^{a}
	(0.42)	(0.021)	(0.015)	(0.67)
Guinea grass	11.30^{c}	0.562^{ab}	0.185^{a}	33.62^{b}
	(0.41)	(0.021)	(0.015)	(0.67)
Rice straw BG297-2	7.42^{b}	0.659^{c}	0.363^{d}	34.66^{b}
	(0.36)	(0.020)	(0.014)	(0.62)
Rice straw BG298-2	6.97^{b}	0.627^{c}	0.321^{c}	33.89^{b}
	(0.35)	(0.020)	(0.014)	(0.61)

Different superscripts per column indicate significant differences ($p < 0.05$).
[1] means not corrected.
[2] means corrected for mineral effect and AN level.
[3] means corrected for AN level.

and the AN level of the substrate is given in table 8. The interaction between feed and the AN level was not significant for these two degradation parameters, which indicates, that for all feeds the maximal value of the degradation parameter is found at the same AN level. The required AN level for the maximum value of degradation parameters is given in table 8 as well as the required AN level to reach 95 % of maximal degradation, which was 88.2 mg AN/l for OMD_{48} and 100.0 mg AN/l for k_1.

The relation between k_2 and the AN level of the substrate was dependent on the feed. The regression equations describing this relation for each feed are given in table 9 as well as the required AN level for maximal k_2 and for 95 % of maximal k_2. The required AN levels for maximum k_2 are lower than the required AN level for the maximal level of the other degradation parameters. To reach 95 % of maximal k_2 guinea grass has a lower AN requirement than the other feeds.

The number of test feeds was too small to test for correlations between feed composition and degradation parameters or required AN levels.

TABLE 8. RELATION BETWEEN DEGRADATION PARAMETERS AND THE AN LEVEL OF THE SUBSTARATE (BETWEEN BRACKETS SEM)

	OMD_{48} (%)	K_1 (%/hr)
Linear regression coefficient ($*10^{-2}$)	43.5 (3.5)	0.969(0.111)
Quadratic regression coefficient ($*10^{-4}$)	−17.5 (2.6)	−0.356(0.085)
Required AN level for maximal degradation (mg/l)	124.3	136.1
Required AN level for 95% of maximal degradation (mg/l)	88.2	100.0

TABLE 9. RELATION BETWEEN K_2 AND THE AN LEVEL OF THE SUBSTRATE FOR THE FOUR TESTFEEDS (BETWEEN BRACKETS SEM)

	Wheat straw	Guinea grass	Rice straw BG 297-2	Rice straw BG 298-2
Intercept	0.141 (0.019)	0.141 (0.019)	0.141 (0.019)	0.141 (0.019)
Linear regression coefficient ($*10^{-3}$)	4.11^{b} (0.99)	1.57^{a} (0.97)	6.53^{c} (1.00)	5.83^{c} (1.01)
Quadratic regression coefficient ($*10^{-5}$)	-2.35^{ab} (0.83)	-1.02^{a} (0.81)	-3.70^{b} (0.89)	-3.49^{b} (0.91)
Required AN level for maximal k_2 (mg/l)	87.4	78.5	88.2	84.0
Required AN level for 95% of maximal k_2 (mg/l)	61.2	45.3	64.2	60.0

Different superscripts per line differ significantly ($p < 0.05$).

Discussion

Urea supplementation of the ration of the donor animals increased the VFA concentration and the NAN concentration of the rumen fluid, which are indications of a higher rate of digestion and a higher microbial growth. However, the higher NAN concentration measured in the rumen fluid may also be due to a higher concentration of feed nitrogen, since intake of the basal ration, when supplemented with urea was also higher (data will be published elsewhere). No relation existed between the pH and the concentration of

VFA's in the rumen fluid nor between the pH and the AN concentration. Why mineral supplementation decreased pH is unknown.

All kinds of supplementation increased the C2/C3 ratio of the rumen fluid. A possible reason could be, that production rates of individual VFA's are affected by a lack of nutrients. There are indications, that the content of lactic acid in the rumen of animals on a sulphur (S) deficient diet is high due to a diminution of the acrylate pathway for conversion of lactate to propionate (Slyter et al., 1986). Other pathways may also be affected by nutrient deficiency. Another reason for the low C2/C3 ratio on the not supplemented diet may be, that relatively more acetate than propionate is absorbed from the rumen in a poor rumen environment compared to a better rumen environment. The phenomenon, that the C2/C3 ratio increases, when supplementary nutrients are added to a low quality ration may also explain the fact reported from Sri Lanka, that the milk fat content increases after supplementation or treatment of rice straw with urea (Ibrahim, personal communication).

Urea supplementation increased the AN content of the rumen fluid. The levels found in this experiment are in line with the levels observed by Erdman et al. (1986), who infused up to 2.1% of urea into the rumen of cows fed on corn meal and cotton seed hulls. The observed AN levels are however much lower than the levels observed by Mehrez et al. (1977) when they supplemented 1% urea to a whole barley grain ration of sheep. The in vitro degradation was positively related to the AN content of the substrate as was also reported by Mehrez et al. (1977) and Erdman et al. (1986). For the feeds studied in our experiment 95% of maximal k_1 and OMD_{48} was reached at AN levels of 88-100 mg/l in the substrate.

No relation between the potential digestibility and the required AN level for maximal k_1 or OMD_{48} could be found in this experiment in contrast of findings of Erdman et al. (1986). This may be due to the fact, that the differences between feeds in k_1 and OMD_{48} were small. The k_2, which is more determining the potential digestibility than k_1 and OMD_{48} for the feeds studied in this experiment was different for each feed. The k_2, represents the rate of degradation of the worst digestible part of the feed. There was an indication, that the required AN level to reach 95% of the maximal k_2 was dependent on the k_2 value of the feed. The amount of feeds tested was however too limited to analyse this relation. The required AN level for 95% of optimal k_2 seemed lower than the AN level required for 95% of optimal OMD_{48} or k_1. Further, there was no effect of supplementary minerals to the donor animals on the k_2. A reason for this may be, that due to lysis of microbes and the decreasing amount of substrate during the later phase of in vitro fermentation the AN and mineral levels in the substrate are less limiting than during the first phase of in vitro fermentation. The required AN level found here is high when compared to data reported by Erdman et al. (1986), who calculated a relation between potential digestibility and required AN level for a number of feeds varying in maximal digestibility from 45 to 80%. The relation they calculated was:

Required AN = $-157.1 + 4.15$ * (potential digestibility) ($R^2 = 0.50$). Assuming a potential digestibility of 50% for all feeds tested in our experiments the required AN level should be 50.4 mg/l, lower than the observed required AN level of 88-100 mg/l. The residual variation of above regression was large, however, making a judgement of this difference difficult.

Mineral supplementation of the donor animals did not increase the NAN level nor the AN level of the rumen fluid. The increased VFA content may however be an indication of a higher microbial activity. This higher microbial activity was also found when the rumen fluid of the animals that were supplemented with minerals was used for in vitro incubation. The effect of the mineral supplementation on the in vitro degradation parameters may be contributed to sulphur, since all other macro elements in the mineral mixture were added in the buffer solution. There could however also be an effect of the micro elements and/or the vitamines, that were added in the mineral mixture. Effects of S addition to supplementary urea are variable (McLennan et al., 1981) and may be dependent on the ratio between rumen available N and rumen available S. S supplementation increased the amount of cellulolytic bacteria and the VFA production in continuous cultures as well as in the rumen of sheep and calves (Slyter et al., 1986).

Although we added a considerable amount of glucose to the substrate (about 15-20% of the

digestible organic matter) an effect of this addition to a urea supplement could not be found. This is in contrast to what Hoover (1986) mentioned in a review about fiber digestion. In vitro as well as in vivo the fiber digestion was depressed by addition of readily fermentable carbohydrates due to preference of microbes for these carbohydrates and a reduction in pH. In the in vitro system we used the pH was buffered however.

The feeds tested here showed variation in solubility and k_2, but much less in k_1 and OMD_{48}.

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