IN VITRO FEED TEST FOR EVALUATION OF ENERGETIC AND PRODUCTIVE VALUE OF STRAW, INDUSTRIAL BY-PRODUCTS AND ALTERNATIVE ENERGY RESOURCES FOR CATTLE FEEDING

J Juul-Nielsen

The Centre for Veterinary and Agricultural Documentation, 15, Bulowsvej, DK-1810, Copenhagan, Denmark

An in vitro fermentation method for screening of the best combination of available feed resources is described and discussed. 6 x 2 roughly ground samples (0.5 g dry matter) are incubated for 0, 5, 20, 44, 68 and 92 hours at 38-39°C in 100 ml buffer (pH 6.8) with 2 ml rumen liquid. Nitrogen, ?phosphorus, Sulphur and chelated microminerals are supplied at a rate of 2% N in the dry matter (DM) and the ratio N:P:S:chelated minerals equal to 10:2:1: 0.25.

It is argued that the fermentation process basically is enzymatic and assuming a first order reaction. The results are calculated from the semilogarithmic equation:

% soluble dry matter = a + b x ln (hours), which is a straight line (r^2 = 0.09-1.00).

Descriptive parameters are : Maximum digestibility (80 h), and rate of fermentation in terms of g soluble DM/kg DM intake in 24 h (V_{24}).

Maximum DM intake in kg/24 h (MDMI) is calculated from :

$$MDMI = BW^{.73}\left(\frac{4.8}{\%\ \text{residue}} + 0.000245\ (V_{24}{}^{-240})\right)$$

and Maximum Energetic value (MEV) in MJ metabolizable energy (ME)/24 h from:

$$MEV = BW^{.73}\ (MDMI \times V_{24} \times 0.020).$$

Results are shown on a variety of feed resources including industrial by-products and silage. The importance of N:P:S:chelated minerals are shown for barley straw, where MEV increases from 0.16 $MJ/BW^{.73}$ to 0.65 $MJ/BW^{.73}$ using urea with chelated minerals (Grindmix 103).

The result. are published at the present stage to stimulate further work at the farm level in the countries where straw and by-products are the major feed for cattle.

Key words: in vitro fermentation technique, ruminants, chelated microminerals, urea, NPS, nitrogen, phosphorus, digestibility, rate of fermentation, straw. silage, straw mix, maximum energetic value

Ruminants or rather the rumen microbes are extremely good at utilizing waste material and plant crop products, which are not utilized by monogastric animals or human beings.

Tilley and Terry made an in vitro fermentation method work in practice in 1963 and their paper (Tilley and Terry 1963) is probably the most cited article during the last 8 years, being cited more than 650 times (Tilley and Terry 1980). Although the method of Tilley and Terry is very often used and cited, the results on digestibility are only one aspect, though an important one, of the energetic and productive value of the feedstuffs under investigation.

The purpose of this paper is to incorporate into an in vitro fermentation feed test as much as possible of our present knowledge concerning ruminant nutrition and physiology, emphasizing the processes in the rumen as described by Kaufmann (1976), Forbes (1977), Church (1975) and Wangsness and Muller (1981), and bearing in mind the need for simplicity as suggested by Minson (1976).

Background Story: Ørskov et al (1980) among many authors,ciearly describe the differences of the rate of degradation among feedstuffs and if two feedstuffs reach the same level of digestibility they may not have the same pattern of fermentation. The fermentation rate can be different. Practical feeding experiments with two such feeds show that the one with the highest rate of fermentation allows for higher levels of production.

Concentrates such as grains and oilcakes are supplied to a roughage ration to obtain higher levels of production. Starch and protein are the main ingredients in concentrates which are highly and rapidly digestible, whereas roughage typically contains cellulose and protein and is said to be only slowly degraded in the rumen.

By nature ruminants are those animals capable of utilizing cellulose rather than starch as their main carbohydrate source. If roughage or industrial waste products have to be used for cattle feeding the following two questions may arise:

1. What is the productive energetic value of a feed resource, fed alone and ad libitum in terms of maximum metabolizable energy/ unit of metabolic bodyweight (MJ ME/$BW^{.73}$)?
2. Is it possible to find other feedstuffs locally, which will supply the major feed resource , and is a mixed ration of these feedstuffs better than each of them fed alone ?

These two questions are combined into the purpose we all need to be aware of, namely:

Which combination of locally available feed resources will give us the highest production of human food from the animals we have, for one reason or another?

For studies on the background of the in vitro fermentation technique, attention should be paid to the reviews by Warner (1965) and Johnson (1966).

Materials and Methods

Tilley and Terry (1963) stated ; "The innoculum of microorganisms is supplied as strained rumen liquor (10 ml), This supplies adequate levels of accessory factors for efficient digestion, such as valeric acid and trace elements, together with protein for bacterial growth", The fermentation in vitro is stimulated to such an extent, that the authors later say : "The final evaluation with animals is essential, as in vitro digestion trials can be a guide only, to the potential, rather than to the realizable value of feed".

If it is not known which essential substance must be added to the feed ration for cattle, it will not be possible to obtain the same pattern of fermentation in vivo as found in vitro with rumen liquor.

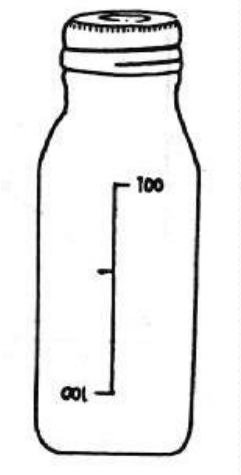
Den Braver and Eriksson (1967) introduced the one stage technique using 1 ml rumen liquor and 96 hours' fermentation supplying the buffer with ammonium phosphate. Later (Den Braver 1974) the method was used to determine metabolizable energy in straw, but in this case urea, magnesium and sulphur were suggested as important supplements both in vitro and in vivo.

The potential of the feed must be in the feed itself or in the feed ration, so the purpose of the rumen liquor should be an easy way of inoculation to establish the microbial basis for fermentation. Thomson et al (1978) and Juul-Nielsen (1978), have found that nitrogen, phosphorous and sulphur have the highest priority for a progressive and even fermentation and the inclusion of chelated microminerals (Grindmix 101) in the feed may increase the rate of fermentation even more. The feed sample is roughly ground in a coffee mill with the purpose of obtaining a representative sample. Compound feeds are not ground.

0.5 g DM is used for incubation in 100 ml phosphate buffer (Den Braver 1969) containing 2 ml rumen fluid and 30 mg of a feed supplement : NPS 180[1] with chelated microminerals Grindmix 101[2] which is normally supplemented to the feed ration.

In Figure 1 the incubation flask used is shown. Centrifugation is not used and hence the quality of the flask is not critical. The closed bottle assures anaerobic conditions and allows only the gas produced during the fermentation to escape.

Figure 1 :
The incubation flask used has a volume of 100 ml and a slit is cut in the rubber membrane in the screw top



6 x 2 feed samples are incubated at 38-39°C and 2 samples are taken out of the incubator after 0, 5, 20, 44, 68 and 92 hours incubation, filtrated and dried to constant weight at 100°C.

[1] *Purchased from K.F.K, Groendalsvej 1, DK-8260 Viby, Denmark*

[2] *Purchased from Grinsted Products, Edwin Rahrsvej 38, DK-8220 Brabrand, Denmark*

Soluble DM at the time (t) is calculated as percent of the DM incubated. The method can be refined, determining organic matter, but only in a few feedstuffs with extraordinarily high ash content is the extra work justified by adding more information to the results based on DM determination alone.

Figure 2:
Barley straw from France (Table 1 no. 12) is tested and the fermentation curve 4 drawn automatically by a hand calculator (Texas Instrument 59) together with the calculated amount of residue at to hours and available digestible DM in 24 hours/ kg DM intake (V_{24})

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IN-VITRO FEEDTEST  16/3-81

SAMPLE:
FRANSK HALM 3/3-81

NOTES:
 Dry substance % 90.85
 500 mg of sample inocc. with
 2 ml of fresh rumen liquid.

Hour     DS %      pH
  0       4.2      6.8
  5       4.2      6.8
 20      17.4      6.9
 44      32.9      6.7
 68      37.3      6.7
 92      45.0      6.7

FERMENTATIONCURVE:

Curvebase at: 9 hour.
DS%= -34 + 17*ln(hours)
R^2= .986

V24(ad mod.JJN):  392

DS-SPLIT %

WSOL FAST SLOW RESD
  4   14   24   58
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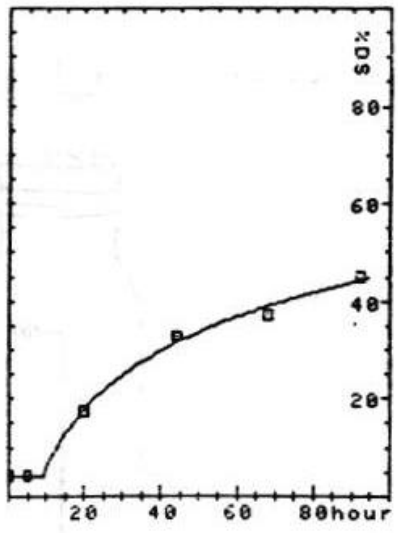
PLOT:



The results are introduced into a programmable hand calculator and the pattern of fermentation is drawn automatically together with the calculated parameters as seen in Figure 2 for barley straw. Our experience shows that the ragtime seen in Figure 2 is unimportant for the conditions in vivo (Donefer et al 1962) except for fermented or processed products such as silages differing in quality.

Calculations: The fermentation process is basically an enzyme reaction as pointed out by Juul-Nielsen (1978),hence the rate of fermentation is influenced by pH (mostly constant due to the buffer in the medium), temperature (specified as the body temperature, 38-39°C), the enzyme concentration and the substrate concentration. The product concentration may be neglected in dilute solutions. When the lag phase is over and the fermentation has started the enzyme concentration is not a limiting

factor. Finally the resulting reaction may be seen as a first order reaction, which depends mainly on the substrate concentration at any time. A semi logarithmic plot of an even and progressive fermentation must show up as a straight line, which enables us to calculate a rate constant and percentage of solubility at any time after incubation.

Dissolved DM (%)

$$\% \text{ dissolved DM} = a + b \times \ln(\text{hours}); \; r^2 > 0.09 \qquad (A)$$

Water soluble DM (%)

Average of values during the lag phase mostly 0 and 5 hours

Total digestible DM

Soluble DM (%) at 80 hours, use equation (A)

Lag phase (hours) 1 = curvebase

Insert % water soluble DM in equation (A) and calculate hours

Rate of fermentation

(g soluble DM/kg feed DM/24 hours) = V_{24}

The rate constant is b in equation (A), but to simulate the interrupted intake of feed, only the first six hours after the fermentation has started are used to calculate the average velocity of the fermentation (suggested by Warner 1956). The average value for six hours is multiplied by four to get fermented DM within 24 hours. Furthermore the water soluble portion is added to arrive at digestible DM (g/kg) within 24 hours.

$$V_{24} = 10 \left(\% \text{ watersoluble} + 4 \times b \times \ln\left(\frac{\text{curvebase} + 6}{\text{curvebase}}\right)\right) \qquad (B)$$

Maximum DM intake (MDMI) (kg/day/BW^{73})

The experimental basis is published by Conrad et al (1964), McCullough (1973), Juul-Nielsen (1978) and Juul-Nielsen (1981), The equations published are modified to make the results independent of bodyweight substituting the liveweight with metabolic bodyweight (BW 73), which is proportional to the rumen volume. Obviously the rumen volume determines the amount of substrate taking part in the enzymatic reactions described in equation (A) and (B), and consequently the disappearance and input of DM/unit of time.

$$MDMI = BW^{.73} \left(\frac{4.8}{\% \text{ residue}} + 0.000245 \, (V_{24} - 240)\right)$$

:-------a------: :------------b-------------:

a: The portion of MDMI which is determined by indigestible DM.eg the DM left over at 80 hours after the start of incubation.
b: The portion of MDMI which is determined by the rate of fermentation (V_{24}) above a basic value estimated to be 240. (Juul-Nielsen 1980 unpublished data).

Maximum energetic value (MEV) (MJ/BW$^{.73}$)

If the total amount of substrate in rumen is known (MDMI), it is possible to calculate the available digestible DM. Assuming 20 MJ (range 19 -21 MW) metabolizable energy/kg digestible DM, the maximum energetic value for maintenance and production is calculated from equation (D)

$$MEV = BW^{.73} (MDMI \times V_{24} \times 0.020) \quad (D)$$

or calculation of maximum productive value (MPV) by subtracting energy for maintenance 0.55 MJ/BW$^{.73}$ from equation (D)

$$MPV = BW\ 73 (ND\ MI \times V_{24} \times 0.020 - 0.55) \quad (E)$$

Results

The results are presented in Figure 3 . Table 1 shows a selection of samples tested through 1980 - 1981.

The information given on the sample sheets (Figure 3) includes description of the sample, the experimental results, % soluble DM and pH. pH is measured to verify that it is above 6.5, showing that the buffer capacity is not exceeded by volatile fatty acid production.

Furthermore the curve is drawn and described by the four parameters: curvebase, semilogarithmic equation, regression coefficient (r^2) and rate of fermentation (V_{24}).

Finally for those not familiar with mathematical expressions the pattern of fermentation is expressed by splitting the curve into four fractions: watersoluble, fast soluble (2.5-20 hours), slow soluble (20-80 hours) and residue (80 hours).

The values shown in Table 1 are used for further calculations of MDMI and MEV which is only valid for unprocessed plant crops or for full feed rations including one or more of the industrial by-products together with straw.

MDMI is also shown in Table 1 and this is a maximum value, but according to practical experiments (Juul-Nielsen 1978; Juul-Nielsen 1980 unpublished data) the values found on the farm are less than 10% below the calculated figures..

MEV reach surprisingly high levels but if these values are used to optimize a feed ration based on barley straw, rice straw or wheat straw, the productive results, milk production or weight gain,will be within 10% of the predicted result.

The importance of nitrogen, phosphorous, sulphur and chelated minerals in the feed is shown in Table 2. Barley straw incubated alone shows a low value on both digestibility and rate of fermentation (V_{24}). By adding 2% non-protein nitrogen, as urea, the digestibility increases to 36% and V_{24} from 176 to 299, and MEV from 0.16 MJ to 0.53 MJ/BW$^{.73}$, which is just enough for maintenance .

Figure 3: 3:
The results from 3 feeds are presented:
a. Rice straw from Indonesia
b. Strawmix produced in Scotland containing sot barley straw, 28% molasses and 22% Premix with NPS, chelated minerals and sugar beet pulp/citrus pulp /soy hulls
c. Maize crop silages produced in Denmark (Table I no.9)
Digestibility: STRAWMIX 59%, Rice straw 55%, Maize silage 45%
V_{24} : STRAWMIX 589, Maize sialge 469, Rice staw 430
MDMI/BW$^{.73}$: STRAWMIX 0.167 Maize silage 0.163, Rice straw 0.134
MEV/BW$^{.73}$: STRAWMIX 1.96, Maize silage 1.53, Rice straw 1.16

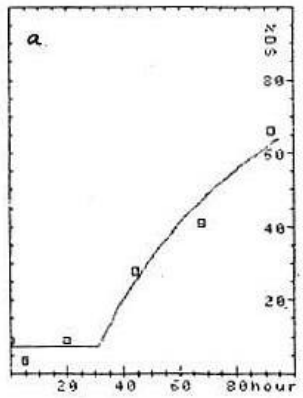
IN-VITRO FEEDTEST 15/6-81

SAMPLE:
RISHALM/INDONESIEN 25/5-81
SKJOLD SEBY

NOTES:
Dry substance % 91.2
500 mg of sample inocc. with
2 ml of fresh rumen liquid.

Hour	DS %	pH
0	9.0	6.6
5	3.5	6.6
20	9.0	6.7
44	27.6	6.9
68	40.8	6.7
92	66.0	6.7

PLOT:



FERMENTATIONCURVE:

Curvebase at 31 hour
DS%=-166 + 50*ln(hours)
R^2= .922

V24(ad mod JJN): 430

DS-SPLIT %

WSOL	FAST	SLOW	RESD
7	0	48	45

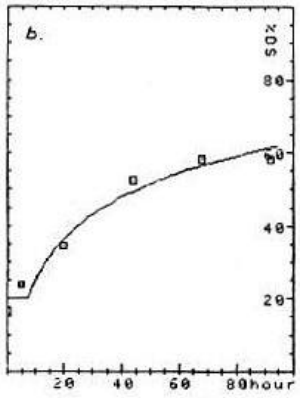
IN-VITRO FEEDTEST 30/3-81

SAMPLE:
STRAA-MIX 50-28-22
NORTRON FS AGRO

NOTES:
Dry substance % 84
500 mg of sample inocc. with
2 ml of fresh rumen liquid.

Hour	DS %	pH
0	16.7	6.7
5	23.8	6.7
20	34.5	6.6
44	52.4	6.6
68	58.3	6.6
92	58.3	6.8

PLOT:



FERMENTATIONCURVE:

Curvebase at 7 hour
DS%= -13 + 16*ln(hours)
R^2= .932

V24(ad mod JJN): 589

DS-SPLIT %

WSOL	FAST	SLOW	RESD
20	16	23	41

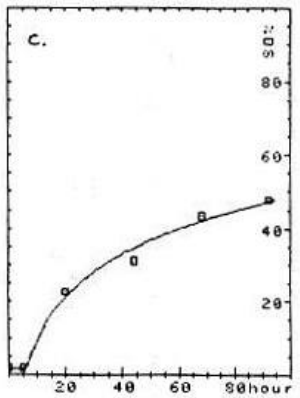
IN-VITRO FEEDTEST 16/2-81

SAMPLE:
MAJSENSILAGE
JØRGEN PEDERSEN NR.ALSLEV

NOTES:
Dry substance % 23.9
1000 mg of sample inocc. with
2 ml of fresh rumen liquid.

Hour	DS %	pH
0	1.7	6.6
5	1.7	6.6
20	22.6	6.6
44	31.0	6.5
68	43.5	6.7
92	47.7	6.5

PLOT:



FERMENTATIONCURVE:

Curvebase at 6 hour
DS%= -30 + 17*ln(hours)
R^2= .955

V24(ad mod JJN): 469

DS-SPLIT %

WSOL	FAST	SLOW	RESD
2	19	24	55

Table 1:

The in vitro feed test applied to different feeding stuffs including the calculation of Maximum Drymatter Intake (MDMI), Maximum energetic Value (MEV) and Maximum Productive Value (MPV)

	Water sol. DM	Digestible at 80 h	Lag phase	Fermentation rate/24 h	Max DM intake	Max. energetic value	Max productive value	Significance
	%	%	h	V_{24} [1]	MDMI [2]	MEV [3]	MPV [3]	r^2
1. Cotton seed cake, Egypt	15	31	5	335	9.3	62.2	7.2	1.00
2. Palm cake	5	44	11	396	12.4	98.2	43.2	0.98
3. Brewers offal, pellets	13	42	14	371	11.5	85.2	30.2	0.89
4. Citrus residues, (best quality)	42	70	10	683	26.9	366.8	-	0.99
5. Soy hulls	8	74	14	619	27.7	343.5	-	1.00
6. Rice hulls	-1	8	5	98	1.7	3.4	-	0.97
7. Whole crop silage, barley (22.9% DM)	13	42	14	373	11.5	86.0	31.0	1.00
8. Whole crop silage, barley (58.2% DM)	20	55	3	657	20.9	274.4	219.4	0.99
9. Whole crop silage, maize (23.9% DM)	2	45	6	469	14.3	134.5	79.5	0.96
10. Whole crop silage, maize (37.6% DM)	4	60	8	589	20.6	242.1	187.1	0.91
11. Hay silage (58.8% DM)	23	71	6	734	28.7	420.7	365.7	0.96
12. Barley straw, France	4	42	9	392	12.0	94.1	39.1	0.99
13. Barley straw, Denmark	2	40	19	308	9.7	59.5	4.5	1.00
14. Rice straw + NPS 180	5	47	15	388	12.7	98.4	43.4	0.98
15. Rice straw without suppl.	5	39	12	345	10.4	72.0	17.0	0.99
16. Barley straw, treated with 4% NaOH	11	55	11	497	17.0	168.6	113.6	0.98
17. Barley straw, Denmark	5	36	12	316	9.4	59.2	4.2	0.95
18. Sugar beet pellets	2	71	6	746	28.9	431.9	-	0.98
19. Sugar beet molasses	84	90	23	880	63.7	1120.1	-	0.41
20 55% straw (17), 25% molasses (19) 15% Beet pellets (18), 5% premix NPS 180	29	58	8	577	19.7	227.2	172.2	1.00
21. 20 Rations calculated from the values of individual feeds	29	58	(19.5)	556	19.2	213.0	152.0	-

[1] g digestible DM/kg DM in 24 h

[2] Maximum DM intake/24 h, kg/$BW^{.73}$ x 100

[3] MJ metabolizable energy /kg $BW^{.73}$ x 100

Table 2:

Production value of straw with various supplements of NPN, minerals and molasses. Based on results from in vitro feed test profiles

	Digestibility % DM	V_{24} [1]	MEV [2]	MPV [2]
1. Barley straw	21	176	0.16	-
2. + 2 % NPN (urea)	36	299	0.53	0.00
3. + 2% NPN + mineral + vitamin mixture (Rouf 1976)	35	308	0.59	0.04
4. + 2% NPN + Grindmix 103	33	339	0.65	0.10
5. + 2% NPN + Grindmix 103 + 10% molasses	34	367	0.76	0.21

In vitro incubation temperature: 38 - 39°C

0.5 - 1.0 g DM/100 ml buffer/2 ml rumen liquid

[1] g digestible DM/kg DM in 24 hours

[2] MJ metabolizable energy/kg $BW^{.73}$

Rouf (1976) added both vitamins and minerals as ordinary salts. The addition of chelated minerals as Grindmix 103, which is Grindmix 101 + magnesium sulphate, resulted in still higher values. The digestibilities do not differ very much once the nitrogen requirements are fulfilled but the rate of fermentation is changed and so are both MEV and MPV.

Discussion

Ruminants utilize a great variety of feed resources most efficiently. The ammonia released in the rumen surplus to the requirements of the microbes is absorbed and partly recycled as urea through the saliva. Furthermore undegraded protein and soluble starch are passed on to the duodenum for enzymatic degradation and absorption.

Ruminants are unique in their ability to utilize cellulose or cell wall material and in a situation of limited food resources, roughage and industrial by-products with cellulose are the only feed available for ruminants.

Through the microbial processes the rumen becomes the most important site of degradation of cellulose and synthesis of protein from non-protein nitrogen (NPN).

The one-stage in vitro fermentation technique has its advantages in the study of requirements for microbial reactions and multiplication especially if cellulose rich material has to be fed (Juul-Nielsen 1978). As much as 80% of the nitrogen may come from urea if the ration fed is based on cell wall material, cellulose or straw (Juul-Nielsen 1981). The minimum is 20% nitrogen from NPN. Starch is not necessary to utilize urea but the urea must be sprayed on to the roughage and not given in the concentrate. Adequate mineral supplementation in a bioavailable form also seems to be important for cellulose degradation (Hunt et al 1954; Chamberlain and Burroughs 1962; Bryant 1973; Kropp et al 1977.

The ready mixed Premix NPS 180 is available in Denmark and was supplied both in the in vitro feed test and in the strawmix ration. The supplement NPS 180 is not

very important, although it is the best we know today. It is more important that the minerals supplied for the in vitro feed test are also available at the farm level.

Results obtained in the laboratory are useful only if they simulate what is possible on the farm. Feedstuff tables may only be useful in the countries where the feedstuff industry is a reliable supplier, and are possibly useless in developing rural areas (Jackson 1980; Preston 1981).

The in vitro fermentation technique described in this paper seems to be simple and suitable for routine purposes. The traditional chemical analysis for feedstuff evaluation need not be carried out except for determination of dry matter, and sometimes nitrogen and ash. The nylon bag technique (Ørskov et al 1980) is not discussed but may be considered for special scientific purposes or in areas where fistulated cows are avail able as the only possible incubator.

Essentially the cow should be fed the same feed as is under test in the nylon bag, to assess the full potential of the feed, this being related to the changes in the microbial flora caused by the feed itself.

Ørskov et al (1980) used 9 samples at a time in the sheep rumen and this may be a major disadvantage if the test is used as a routine feed test, testing 20 - 30 feeds or feed rations per week. At least 30 fistulated sheep may be needed for this number of tests.

The parameters and methods of calculation developed during the last 5 years are presented at the present stage, although further work needs to be done in comparing the predicted results obtained under practical farm conditions.

The equation:% digested DM=a + b x ln (hours) can be transformed to the form suggested by Ørskov et al (1980), but our experience is that the presentation shown in Figure 3 can be understood by the layman .

The values for MDMI and MEV are left out in Figure 3 and should only be calculated during the formulation of the feeding scheme. MDMI and MEV are extremely useful in the case of feed rations aiming at using maximum roughage or by-products and saving concentrate at high levels of production.

Table 1 shows examples of feed tests chosen out of approximately one thousand fermentation curves on feeds investigated on request from both farmers and feed mills. The results from individual feeds can be weighted according to the percentage of inclusion in the feed rations and the sum of weighted figures compared to the in vitro test on the mixed ration are surprisingly close.

Barley straw (17)[1], sugar beet pellets (18) and sugar beet molasses (19) are the feedstuffs used in the strawmix ration (20). The NPS 180 is fully watersoluble and the sum weighted according to the percentage given for strawmix (20) is shown in (21). Similarities are more impressive than the differences.

The digestibility in this in vitro system is additive in combinations. The same is true for water soluble DM. As can be seen from Table 2 the sensitivity of the system is high. Small differences in the feed eg variety, time of harvest, quality of silage making, fat content in concentrates, are reflected in changes of the rate of fermentation V_{24} as seen in both Table 1 and Table 2.

It is certain that more experiments with animals under different conditions are needed to know if the principles for the calculations can be used in all situations. The

[1] *Reference number, see Table 1*

sheep and the goat are ruminants too but they may not fit fully into the equation (A) for MDMI. The figures calculated are too high, but the ranking order seems to be the same both in vitro and in vivo. Hence the overall purpose in finding the best combination of available feed resources for production of human food can be achieved for these animals too.

The in vitro feed test seems to give the answer to some important questions for planning and optimizing cattle feeding and production. But the requirements for the animal itself must be considered seriously according to common practice. Vitamin A is one essential nutrient which must be supplied.

Intoxications may occur due to mimosine in *Leucaena leucocephala* (IpilIpil) (Meulen et al 1979) and due to HCN in cassava (Ngarmsak 1978), if fed as the only feed, but fed in combinations with straw, the toxic symptoms can be eliminated.

The physical structure of the feed is a significant question too. The balance between maximum DM intake and particle size is a critical point, which is optimized for cattle, when the particle size is 2-10 cm (Sudweeks et al. 1979; Dewysen and Vanbelle 1978; Welch and Smith 1978).

The need for by-pass protein (undegraded nutrients) (Ørskov et al 1980) is still a questionable subject which is important in the strawmix system only in the case of very high milk production of more than 0.4 kg milk/ $BW^{.73}$. The need for feeds with undegradable nutrients has to be fulfilled either by natural feeds such as groundnut cake, fat or heat protected protein or, what is also possible, by stimulating the microbial synthesis of protein.

Conclusion

The in vitro feed test described and discussed is based on the findings of Tilley and Terry (1963) and Den Braver and Eriksson (1967), using 0.5 g feed DM/100 ml buffer solution (pH 6.8)/2 ml rumen liquid. Following the pattern of fermentation, 2 samples are taken out of the incubator (38-39°C) at 0, 5, 20, 44, 68 and 92 hours after the start of the incubation. 3 mg of a chelated mineral supplement NPS 180 is added. The nitrogen level is 2% on a DM basis, NPS ratio is 10:2:1. Total digestibility (80 hours), watersoluble DM before fermentation initiates after the lag phase (0 - 20 hours), and the fermentation curve: (% digested DM= a + b x ln(hours)) are parameters used to calculate maximum DM intake (MDMI), maximum energetic value (MEV) and available nutrients in 24 hours (V_{24}). The calculated results are related to metabolic bodyweight ($BW^{.73}$) assuming ad libitum feeding of mixed feed rations, rich in straw or cell wall material. The method is used routinely to help both feedmills and farmers to maximise utilization of roughage and at the same time save concentrate at high levels of production. In developing countries the method may be used for screening of all feed resources locally available. The sensitivity is high and the effect of interaction between feeds is used to predict the maximum level of production, using urea in the range of 20 - 80% of the nitrogen in the ration.

The in vitro test is running routinely as a customer service for practical farmers, but also the feed test is applied to feedstuffs to be included in concentrates or compound feed for ruminants or to judge the quality of silage and strawmix rations prepared under farm conditions.

References

Bryant P 1973 Nutritional requirements of the predominant rumen cellulolytic bacteria Federation Proceedings 32(3):1809-1813

Chamberlain C C , Burroughs W 1962 Effect of fluoride, magnesium and manganese ions on in vitro cellulose digestion by rumen microorganism" Journal of Animal Science 21:428-432

Church D C 1975 Digestive physiology and nutrition of ruminants Metropolitan Printing Comp Portland Oregan USA 1:266-279

Conrad H R, Pratt A D 6 Hibbs J W 1964 Regulation of feed intake in dairy cows I Change in importance of physiological factors with increasing digestibility Journal of Dairy Science 47:54-62

Den Braver E & Eriksson S 1967 Determination of energy in grass hay by in vitro methods Lant brukshogskolans annaler 33:751-756

Den Braver E 1969 Determination of metabolizable energy in dried forage crops Lantbrukshogskolana annaler 35:921-934

Den Braver E J 1974 Determination of metabolizable energy in straw for ruminants by in vivo and in vitro methods Swedish Journal of Agricultural Research 4:5 }59

Dewysen A,G Vanbelle H 1978 How chopping improves grass silage intake by sheep and heifers . Proceedings of the 7th General Meeting of the European Grassland Federation. Merelbeke Belgium 6:19-6.26

Donefer E, Lloyd L E & Crampton E W 1962 Prediction of the nutritive value index of forage fed chopped or ground using an in vitro rumen fermentation method Journal of Animal Science 21:815-818

Forbes J M 1977 Interrelationships between physical and metabolic control of voluntary food intake in fattening,pregnant and lactating mature sheep: A Model Animal Production 24:91-101

Hunt C H, Bentley O G, Herschberger T V & Cline J H 1954 The effect of carbohydrates and sulfur on vitamin B synthesis, cellulose digestion and urea utilization by rumen microorganisms in vitro Journal of Animal Science 13:570-580

Jackson M G 1980 Ways and means of improving livestock production and productivity in the Danida Assisted integrated rural development project Noakhali district Bangladesh

Johnson R R 1966 Techniques and Procedures for in vitro and in vivo rumen studies Journal of Animal Science 25:855-875

Juul-Nielsen J 1978 How to include large amounts of straw in fairy rations and to maximise the productive feeding value of the straw portion Report on the 4th Straw Utilization Conference Oxford 30 November and I December

Juul-Nielsen J 1981 Nutritional principles and productive capacity of the Danish Strawmix system for ruminants Presented at the second seminar on Maximum Livestock Production on Minimum Land Bangladesh Agricultural University Mymensingh February 2-5

Kaufmann W 1976 Influence of the composition of the feeding frequency on pH-regulation in the rumen and on feed intake in ruminants Livestock Production Science 3:10 }114

Kropp J R, Johnson R R, Hales J R & Owens P N 1977 Microbial protein synthesis with low quality roughage rations Isonitrogenous substitution of urea for soybean meal Journal of Animal Science 46(41:837-843

McCullough M E 1973 Optimum feeding of dairy animals for meat and milk The University of Georgia Press Athens USA

Meulen U, Struck S, Schulke E & El Harith E A 1979 A review of the nutritive aspects of *Leucaena leucocephala* Tropical Animal Production 4:113-126

Minson D J 1976 Relation between digestibility and composition of feed Miscellaneous Papers Landbowhoageschool Wageningen 12:101-114

Ngarmsak S 1978 Cassava-rice straw complete rations with supplemented urea for dairy cattle I The digestibility and microbial protein synthesis of the cassava-rice straw complete rations with supplemented urea in vitro Thesis 1-86 Postgraduate Course in Physiology of Animal Nutrition Veterinary Faculty for FAO Fellows Royal Veterinary and Agricultural University Copenhagan Denmark

Ørskov E R, Hovell F D DeB 6 Mould F 1980 The use of the Nylon Bag Technique for the evaluation of feedstuffs Tropical Animal Production 5:195-213

Preston T R 1981 Recommendations from the Second Seminar on Maximum Livestock Production from Minimum Land Bangladesh Agricultural University Mymensingh February 2-5

Rouf A M 1976 Effect of different levels if rice gruel, straw and protein or urea on the digestibility of straw and nitrogen utilization by rumen microorganisms in vitro Thesis from Veterinary Faculty for FAO Fellows Copenhagen 1-33
Sudweeks EM Ely L O & Sisk L R 1979 Effect of particle size of corn silage on digestibility and rumen fermentation Journal of Dairy Science 62(2):292-296
Thomsen L, Moller H & Vibe E 1978 Feeding systems based on untreated straw World Review of Animal Production 14(3):17-27
Tilley J M A & Terry R A 1963 A two-stage technique for the in vitro digestion of forage crops Current Contents/Journal of the British Grassland Society 18:104-111
Tilley J M A & Terry R A 1980 A two stage technique for the in vitro digestion of forage crops Current Contents/Agricultural Biology and Environmental Sciences 15:16
Wangsness P J 6 Muller L D 1981 Maximum forage for dairy cows: Review Journal of Dairy Science 64:1-13
Warner A C I 1956 Criteria for establishing the validity of in vitro studies with rumen micro organises in so-called artificial rumen systems Journal of General Microbiology 14:73}748
Welch J G 6 Smith A M 1978 Particle sizes passed from rumen Journal of Animal Science 46(1): 309-312

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