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Some aspects of the assessment of plant cell-wall constituents by the van Soest method

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Feedstuffs of vegetable origin ingested by humans, as well as by animals show a relatively rigid structure due to the membranaceous plant cell walls which separate the contents of the cells from one another.

These membranes consist of substances, whose digestibility is poor (such as lignin) or medium (such as cellulose, hemicelluloses or cutin). Moreover they can inhibit the action of digestive enzymes on the much more easily digestible cell contents. The digestibility of feedstuffs, and hence, their nutritive value, is very much related to the importance of the plant cell-wall and to its composition.

For more than a century, many investigations have been undertaken to devise analytical methods enabling to quantify with adequate accuracy the influence of vegetable cell-walls on the digestive utilisation of feedstuffs.

Presently the crude fibre method which has been developed over a century ago by researchers at the Weende station is generally used everywhere, but its limitations have been underlined several times.

Among the various methods which have been suggested as an alternative, the van Soest procedure, which was developed almost fifteen years ago is more and more widely used because it enables the determination of the share of the various plant cell-wall constituents, and this, relatively simply and rapidly.

This method is based on the use of detergent solutions, which, after reaction as a residue have the vegetable plant cell-wall (NDF residue in case of neutral detergent: van Soest 1967) or a ligno-cellulosic fraction (ADF residue in case of acid detergent: van Soest 1963). It is subsequently possible to determine the lignin and cutin fractions by using the ADF residue. Cellulose and hemicelluloses are evaluated by difference (diagram 1).

This technique was developed originally for forage samples. Several variations have been suggested in order to adapt it to concentrates, whose cell-wall composition is much more variable (van Soest 1969) and essentially the content of reserve carbohydrates is generally higher.

In this review, we have examined the feasibility of using this method to determine the cell-wall constituents in feeds, more especially in concentra-

tes, by using a semi-automatic measurement equipment.

Filtration problems

In this method, hydrolysis by detergents (neutral or acid), is followed by a filtration in order to separate the insoluble residue from the filtrate.

But when using a neutral detergent, some types of feeds can raise filtration problems: for example, cereals, concentrated compound feeds, maize silages.

In these cases, clogging of sintered glass crucible can take place. It is sometimes possible to overcome this by using a reverse flow of air, and such device is provided for on the Fibertec System.

Nevertheless, this device is not efficient enough for feedstuffs showing a very high starch content. In this case, it is necessary to use a technique recently suggested by Robertson and van Soest (1977) in which a heatstable alpha-amylase is added during the se-

Table 1

Effect of Use of an Amylase

| (% dry matter) | % starch | % Neutral Detergent Fibre | |
|-------------------|----------|---------------------------|--------------|
| | | Without Amylase | With Amylase |
| Concentrate No. 1 | 36 | 18.1 | 16.8 |
| Concentrate No. 2 | 15 | 29.3 | 23.7 |
| Concentrate No. 3 | 11 | 42.4 | 39.9 |
| Concentrate No. 4 | 16 | 38.1 | 28.8 |
| Maize Silage | | 71.9 | 69.7 |

Table 2

Effect of Filtration Speed after extraction on Neutral Detergent Fibre (NDF)

| (% dry matter) | Rapid Filtration | Slow Filtration |
|-------------------|---------------------|---------------------|
| | (less than 10 min.) | (more than 10 min.) |
| Concentrate No. 1 | 14.2 | 21.9 |
| Concentrate No. 2 | 18.8 | 20.5 |
| Concentrate No. 3 | 30.7 | 32.3 |
| Concentrate No. 4 | 30.9 | 32.4 |
| Maize Silage | 51.8 | 56.5 |

Table 3

Effect of Neutral Detergent hydrolysis on the lignocellulose residue (ADF)

| (% dry matter) | ADF from NDF residue | ADF direct |
|-------------------|----------------------|------------|
| Barley | 6.7 | 7.4 |
| Maize | 2.5 | 3.0 |
| Lucerne meal | 24.8 | 32.0 |
| Copra cake | 32.0 | 33.0 |
| Lucerne hay | 33.6 | 34.2 |
| Concentrate No. 1 | 15.3 | 15.5 |
| Concentrate No. 2 | 14.6 | 15.1 |

Table 4

Reproducibility of the two lignin methods

| (% dry matter) | H ₂ SO ₄ 72 % | KMnO ₄ |
|----------------|-------------------------------------|-------------------|
| | Concentrate | 1.3— 1.4 |
| Hay No. 1 | 9.5— 9.7 | 11.0— 12.8 |
| Hay No. 2 | 13.5— 13.7 | 12.4— 13.0 |
| Silage No. 1 | 3.4— 3.2 | 0.7— 1.7 |
| Silage No. 2 | 1.9— 2.0 | 2.5— 2.6 |

Table 5

Comparison of analytical results between manual and Fibertec

| (% dry matter) | NDF | | ADF | |
|----------------|--------|----------|--------|----------|
| | Manual | Fibertec | Manual | Fibertec |
| Hay No. 1 | 54.2 | 55.1 | 52.9 | 52.9 |
| Hay No. 2 | 74.2 | 74.0 | 59.3 | 59.1 |
| Maize Silage | 57.8 | 55.5 | 30.3 | 31.0 |
| Faeces | 65.3 | 65.6 | 52.5 | 52.3 |

3). This deviation in the results can lead to important errors in the prediction of the nutritive value of such feeds as the pectic substances are practically completely digestible unlike other plant cell-wall constituents.

the order of 1 per cent for NDF or ADF and of 4 per cent for lignin when determinations were repeated five times.)

Up till now the van Soest method seemed to offer only a slight improvement in precision as compared to the crude fibre method. In fact it has been used almost exclusively for forage samples, in other words for those plants belonging to two major groups (graminaceous and leguminous) in which the composition of fibre (hemicelluloses, cellulose and lignin) fall within narrow limits.

But the use of this method for concentrates which is made possible by certain modifications of the original method should lead to results qualitatively superior to that yielded by the crude fibre method when predicting the nutritive value of concentrates.

As a matter of fact these feeds contain plant tissues of different organic plant parts and belonging to a great number of plant families having cell-wall contents largely variable.

cond half hour of the hydrolysis. The NDF residues obtained after amylase addition led to lower values than a classic hydrolysis generally when the samples have a very high starch content (Table 1) but it can happen that filtration cannot be performed quickly enough (within less than ten minutes after the end of the hydrolysis). In this case, the NDF values are generally too high and not reproducible (Table 2) because of likely surcharges of the residue in form of substance forming a gel (starch or pectin).

Interest of successive neutral and acid extraction procedures

According to the original van Soest method the hemicelluloses content is determined by a difference between the neutral and acid fibre values which are tended on two different samples of the same substance. Other investigators (Bailey and Ulyatt 1970), have nevertheless shown that the neutral detergent does solubilize practically entirely the pectic substances whereas the acid detergent solubilizes only about half of them.

Consequently a hydrolysis in acid detergent solution of the neutral detergent fibre should result in a lower ADF content than the one which is obtained by direct hydrolysis of the sample in the case of feeds showing a high pectic content such as beet pulp (Table

Choice of a lignin determination method

Van Soest suggested two methods to determine the proportion of lignin in feedstuffs, one using 72 per cent sulphuric acid, the other one a buffered permanganate solution. The first of these methods results from the investigations of Norman and Jenkins (1935) and of Ellis et al (1946). Further developed by Jarrige (1961) and Gaillard (1966), in their methods for separating plant cell-wall constituents.

It is more reproducible than the second one (Table 4) and is more frequently used in research laboratories. Finally it is the only method which has been approved by AOAC (1975).

Conclusion

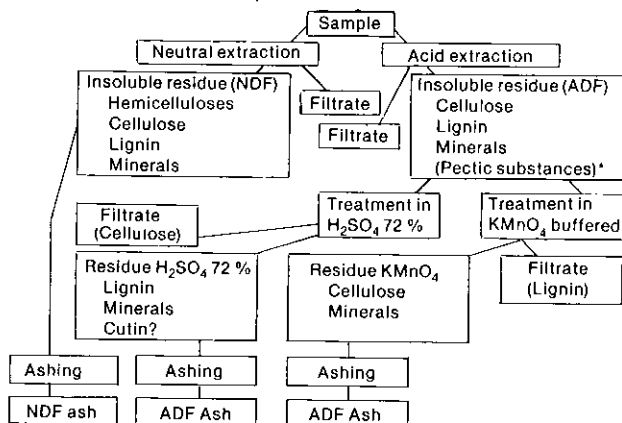
In conclusion this study has led to the suggested determination procedure shown on diagram 2. This method consists of using an amylase with the feedstuffs high in starch content, the sequential neutral and acid hydrolysis on the same sample and the determination of lignin by reaction with 72 per cent sulphuric acid. This procedure can be performed manually or semi-automatically on the Fibertec System both giving comparable results (Table 5).

Furthermore, the reproducibility of the method when using the Fibertec System is good (variation coefficient in



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DIAGRAM I
Principle of the Van Soest method



Van Soest 1967

1. Van Soest (1963) 2. Van Soest (1968) * Bailey et Ulyatt (1970)

Determinations:

Hemicelluloses = NDF - ADF

Cellulose = (ADF - Residue H₂SO₄ 72% (1)) or (Residue KMnO₄ - ADF Ash (2))

Lignin = (Residue H₂SO₄ 72% - ADF Ash (1)) or (ADF - Residue KMnO₄ (2))

DIAGRAM II
Method of operation of semi-automated analysis

