A New Analytical System for Feed Evaluation

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Proximate analysis of feed stuffs (Weende system) has been widely used in many countries. But, in this system, it has long been recognized that fraction of carbohydrates and lignin is imcomplete with respect t othe chemical characterization and nutritive availability.

Numerous chemical and nutritional studies have been attempted[7,10,12] to develop a new system in place of the proximate analysis.

Recently, the study of detergent fiber[9,10] has developed rapidly and its achivements have become a centre of attraction. However, more biological methods using various enzymes have been investigated by Abe et al.[2,3,4] A new enzymatic system developed by the author will be presented in this paper.

Method of enzymatic analysis

Fig. 1 shows the fractionation of feed organic matter by successive treatments with enzymes. Enzymes used, their sources (microorganisms) and commercial supplier were; α-amylase (*Bacilus subtilis*), Wako Pure Chemical Ind. Ltd., Doshu-cho, Osaka, Japan; pronase E (*Streptomyces griseus*), Kaken Kagaku Co. Ltd., Tokyo; Cellulase "for feed analysis" (*Trichoderma viride*), Kinki Yakult Co. Ltd., Nishinomiya, Japan.

The α-amylase was applied to the starch rich samples such as grains, brans and corn silages. A ground sample (0.5 g) was heated with 20 ml

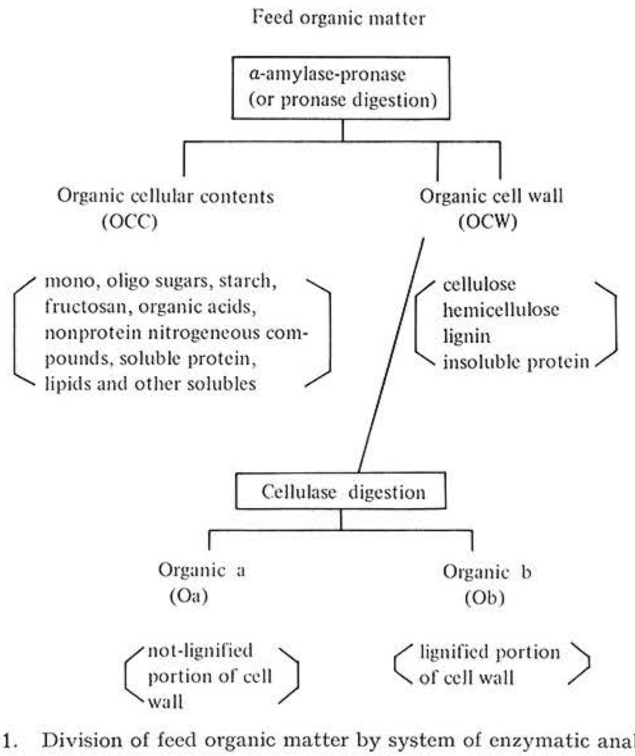

Fig. 1. Division of feed organic matter by system of enzymatic analysis

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of water in a 100 ml Erlenmeyer flask to gelatinize starch. After cooling, 20 ml of 0.005% (W/V) α-amylas esolution (acetate buffer, pH 4.7) was added, the mixture was incubated at 40°C for 16 hr (5.00 pm–9.00 am) with continuous shaking to hydrolyze starch, and was then filtered through filter paper. Residues were subjected to digestion by 0.02% (W/V) pronase (pH 7.4 phosphate buffer, containing 0.35 ppm calcium acetate). Residues were washed into 50 ml polystyrene tubes with approximately 45 ml of pronase solution from a polyethlene wash bottle. In the case of starch-less samples such as grass hays, a ground sample was weighed into 50 ml polystyrene tube directly and pronase solution was added. The suspension was incubated with continuous shaking for 16 hr at 40°C, and then filtered through filter paper. Residues were washed with water and acetone.

A residual dry matter and organic matter was measured. The fraction of residual organic matter was designated as organic cell wall (OCW). The fraction of organic matter digested by α-amylase and pronase was termed organic cellular contents (OCC), and was calculated as feed organic matter (OM) minus OCW.

In another experiment, samples corresponding to 0.3 g of cell wall (CW, residual dry matter obtained by pronase treatment) were treated with α-amylase-pronase or pronase alone in the same manner as described in OCW determination. Residue on the filter paper after pronase digestion, that is CW, was transferred to a 50 ml polystyrene tube with approximately 45 ml of 1.0% (W/V) cellulase solution (pH 4.0 acetate buffer) from a polyethylene wash bottle. Cellulase hydrolysis was performed at 40°C for 4 hr. Residual organic matter was measured, and this fraction was designated as organic b (Ob). The fraction of OCW digested by cellulase hydrolysis was termed organic a (Oa), and was calculated as OCW minus Ob. Thus feed OM was divided into three fractions, OCC, Oa and Ob by enzymatic analysis.

Table 1. Digestibilities (%) of various fractions obtained by enzymatic analysis

Feed	Animal	OM		OCW	
		OCC	OCW	Oa	Ob
Hay (orchard grass early cut)	Sheep[4]	79.8	63.7	98.7	36.2
Hay (orchard grass late cut)	Sheep[4]	69.7	52.5	98.3	37.7
Hay (alfalfa)	Goat	84.1	37.6	86.2	22.3
Corn silage	Sheep[2]	86.9	53.1	91.2	34.6
Formula feed	Pig	93.4	24.6	76.0	6.1

Nutritive characterization of each fraction and their application for feed evaluation

Table 1 shows *in vivo* digestibilities of various fractions obtained by enzymatic analysis. Digestibilities of OCC were higher than those of OCW, and digestibilities of Oa were higher than those of Ob. It is concluded that OCW of feed samples can be divided by cellulase hydrolysis into two fractions remakably differing in digestibility.

Digestibilities of OCW must be influenced by the relative proportion of Oa and Ob in OCW fraction. Actually, it was reported that a negative correlation was obtained between OCW digestibility and Ob content in OCW of hays.[2]

Table 2 shows the correlation between the whole amount and digested amount of each fraction in the case of hay. Highly significant correlation ($P<0.01$) was found between the amount of OCC and that of digested OCC, but there was no such relationship in the case of OCW fraction. It is concluded that OCC fraction is nutritively uniform, but OCW is not. These results were similar to the data obtained by Van Soest[8] who applied a fractionation scheme based

Table 2. Relationships between the contents and digested amounts (with sheep) of each fraction obtained by enzymatic analysis of grass hays[4]

Fraction	Correlation coefficient	Regression equation
OCC	0.995**	$Y=0.968\ X-6.6$
OCW	0.109	
Oa	0.996**	$Y=1.122\ X-3.3$
Ob	0.978**	$Y=0.480\ X-4.0$
OCC plus Oa	0.997**	$Y=1.033\ X-10.1$

X: Contents % DM Y: Digested amounts % DM
** $p<0.01$

on a neutral detergent analysis.

There were high correlations ($P<0.01$) between the contents of total and digestible fractions of both of Oa and Ob. As a consequence, OCW of hay which has no nutritive uniformity can be divided by cellulase hydrolysis into two fractions, each of which is nutritionally uniform. OCC has either high or complete availability and nutritive uniformity. From tables 1 and 2, it is considered that fraction of Oa has similar properties with those of OCC. A high correlation was obtained between total "OCC plus Oa" and digestible portions of" OCC plus Oa." From these results, it appears that high availability and nutritive uniformity can be extended beyond OCC to "OCC plus Oa."

In the case of temperate grass hay, it may be possible to calculate the digestible organic matter (DOM) from the content of "OCC plus Oa" and Ob, and their regession equations shown in the Table 2, because DOM is composed of digestible OCC, digestible Oa and digestible Ob. In addition, it is recognized that the content of "OCC plus Oa" in feed dry matter brings about a useful information to the nutritive evaluation of feedstuffs of cattle (Fig. 2).[6] There was a highly significant correlation between "OCC plus Oa" (DOM determined by the enzymatic method) and *in vivo* DOM of 62 feeds such as ingredient of formula feed, silages, hays, rice straw and commercial formula feeds for dairy cattle.

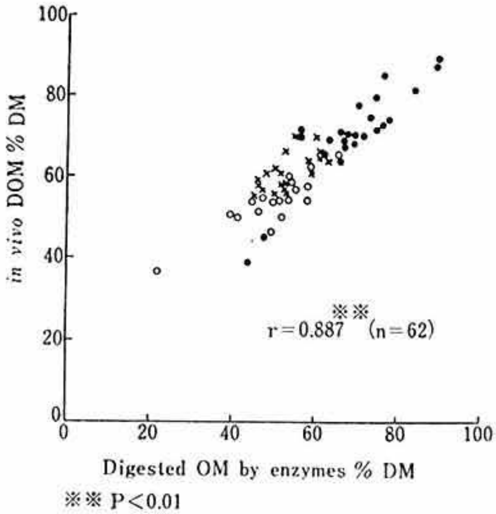

Fig. 2. Correlation between *in vivo* DOM and digested OM by enzymes (OCC plus Oa) of various feeds for cattle[6]
● Ingredients of formula feed and formula feeds for dairy cattle (commercial feeds), × Silages (corn silages, grain sorghum silages and barley silages), ○ Hays and straw (orchardgrass hays, alfalfa hays and grass dominant hays and rice straw)

Attempt to tabulate the feed composition based on the combination of enzymatic analysis and chemical analysis

The organic matter of feed stuffs was divided into two major fractions, OCC and OCW, by enzymatic analysis. The first fraction, OCC, contains substances which can be digested by animals (Fig. 1).

The next interesting problems concerning OCC fraction is its chemical composition, so that the author attempted to tabulate the feed composition based on the combination of enzymatic analysis and chemical analysis. There are many defects in the current system of proximate analysis (Weende system), particulally with the crude fiber determination and calculation of NFE in respect to the classification of carbohydrates and lignin.

It is necessary to separate the carbohydrates into two fractions, such as nonstructural carbohydrates and structural ones. In the new system, OCW is referred to the fraction containing structural carbohydrates and lignin. Nonstructural carbohydrates of feed stuffs are composed of various substances differing in chemical properties.

However, from the nutritional point of view, they can be regarded as one group, because they are completely digested.[1,8] Thus the author proposed a new term, nitrogen and cell wall free extracts (NCWFE), as the nonstructural carbohydrates group: NCWFE=OCC−(crude protein in OCC+crude fat in feed dry matter). Crude protein in OCC can be calculated from the total crude protein of feed and the regression equation shown in Table 3.[6] Crude fat was determined by the usual method of extraction with ethyl ether.

Table 3. Relationships between crude protein percent in dry matter (X) and crude protein percent in OCC (Y) of various feeds group[6)]

Feed group	n	r	Regression equation
Grain, oil meal and formula feed (a)	47	0.994**	Y=0.919 X−0.2
By-product feed (b)	14	0.974**	Y=0.849 X−1.1
Forage and silage (c)	88	0.982**	Y=0.910 X−1.1
Straw (d)	5	0.997**	Y=0.726 X−0.4

**P<0.01

(a) : corn grains, barley grains, wheat grains, oat grains, soybean meal, coconut meal, safflower meals, cottonseed meal, kapok meal, peanut meal, rape seed meal and 14 commercial formula feeds for dairy cattle,

(b) : defatted rice bran, wheat bran, corn gluten feed, shoyu cake, citrus pulps, beet pulp, cocoa residue, sugar cane top, sugar cane bagasse, corn cob, rice hull, cottonseed hull and soybean hull

(c) : rye, oat, sweet sorghum, orchardgrass, timothy, italian ryegrass, tall fascue, meadow fascue, bahia grass, rhodes grass, finger millet, fall panicum, makarikari grass, green panic, alfalfa, ladino clover, red clover, corn silages, barley silages and grain sorghum silages

(d) : rice straw, barley straw, wheat straw, oat straw and soybean straw

Table 4. Contents (% DM) of nonstructural carbohydrates, NFE and NCWFE, and their mutual relationships[5)]

Feed	Mono, oligo saccharides	Starch	Total nonstructural carbohydrates (1)	NFE (2)	NCWFE (3)	$\frac{(1)}{(2)} \times 100$	$\frac{(1)}{(3)} \times 100$
Corn	2.2	66.1	68.3	82.7	71.8	82.6	95.1
Barley	3.2	56.3	59.5	77.6	62.5	76.7	95.2
Wheat bran	6.7	21.7	28.4	62.4	31.1	45.5	91.3
Soybean meal	11.8	1.5	13.3	33.3	19.2	39.9	69.3
Grass hay *1	7.6*2	—	7.6	46.1	7.5	16.5	101.3

*1 : Timothy 50% and orchard grass 50%
*2 : Water soluble carbohydrates (containing fructosan)

Table 5. Digestibilities (%) of nonstructural carbohydrates, NFE and NCWFE

Feed	Animal	Mono, oligosaccharides	Starch	NFE	NCWFE
Hay (alfalfa)	Goat	92.4	100.0	67.0	89.3
Hay (orchard grass)	Sheep	95.7*1	—	56.3	78.3
Corn silage	Sheep	—	100.0	74.2	92.3
Mixed feed*2	Goat	96.4	100.0	77.3	94.3
Formula feed	Pig	97.1	99.8	86.6	99.3

*1 : Water soluble carbohydrates (containing fructosan)
*2 : Alfalfa hay 2 : formula feed 1

Table 6. Composition (% DM) of feeds based on the proximate analysis

Composition	Corn	Soybean meal	Wheat bran	Rice straw	Alfalfa hay
OM	98.5	93.3	94.5	80.5	88.7
Crude protein	9.8	51.5	17.6	4.5	18.3
Crude fat	4.4	2.0	5.0	1.9	2.0
Crude fiber	1.6	6.5	9.5	35.7	30.4
NFE	82.7	33.3	62.4	38.4	38.0

Table 7. Composition (% DM) of feeds based on the detergent analysis

Composition	Corn	Soybean meal	Wheat bran	Rice straw	Alfalfa hay
OM	98.5	93.3	94.5	80.5	88.7
OCC/NDF	84.9/13.6	78.6/14.7	51.5/43.0	12.9/67.6	38.7/50.0
ADF	2.9	9.0	14.4	43.1	37.1
ADF-lignin	0.2	0.3	3.4	5.8	8.4

NDF : Neutral detergent fiber, ADF : Acid detergent fiber

Table 8. Composition (% DM) of feeds based on the combination of enzymatic analysis and chemical analysis (the new system)

Composition	Corn	Soybean meal	Wheat bran	Rice straw	Alfalfa hay
OM	98.5	93.3	94.5	80.5	88.7
OCC/OCW	85.5/13.0	71.5/21.8	51.4/43.1	8.0/72.5	39.4/49.3
Crude protein in dry matter	9.8	51.5	17.6	4.5	18.3
Crude protein in OCC	9.3	50.3	15.3	2.9	15.8
Crude fat	4.4	2.0	5.0	1.9	2.0
NCWFE	71.8	19.2	31.1	3.2	21.6
OCC plus Oa	88.8	75.8	68.5	18.5	51.2
Ob	9.7	17.5	26.0	62.0	37.5

Crude protein in dry matter: total crude protein

Using this new term, a composition of feed OM is expressed in the following way: crude protein in OCC, crude fat, NCWFE and OCW. Table 4 shows the content of nonstructural carbohydrates, NFE and NCWFE and their mutual relationships of various feeds.[5] The ratio of nonstructural carbohydrates to NCWFE was always higher than that to NFE.

Table 5 shows the digestibilities of nonstructural carbohydrates, NFE and NCWFE. Higher digestibilities of NCWFE than those of NFE are shown remarkably in the table. These results suggest that NCWFE is superior to NFE for the expression of nonstructural carbohydrates fraction in feed table. Tables 6, 7 and 8 show the feed composition based on a proximate analysis, detergent analysis and the combination of enzymatic analysis and chemical analysis (the new system), respectively.

It can be concluded that the new system offers more precise and wide imformation in regard to the nutritive value of feeds.

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