

Prediction of apparent digestibility of hays from natural pastures of the Northeast region of Portugal

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Abstract

In the Northeast region of Portugal hays from natural pastures are traditionally used as the main feed during the period of drought and the evaluation of its nutritive value is essential. The aim of this study was to predict the apparent organic matter digestibility (OMD) of 21 hays from natural pastures based on chemical composition, pepsin-cellulase solubility of organic matter (OMS) and *in vitro* organic matter digestibility.

Chemical composition of hays showed a wide range of values and acid detergent lignin (ADL) was the component that presented the highest variation (CV = 18.4%). The OMD varied between 516 and 658 g/kg OM. The best single OMD predictor was OMS (RSD = 2.8%, $R^2 = 0.52$, $P < 0.001$). Using multiple regression to predict OMD, the variables included in the model were OMS and ADL (OMD = $21.51 + 0.94 \text{ OMS} - 0.83 \text{ ADL}$; RSD = 2.75%; $R^2 = 0.54$; $P < 0.0001$). According to principal component analysis (PCA) hays were divided in 3 groups and the multiple regression established for the larger group of hays (n=10) was: OMD = $4.13 + 0.85 \text{ OMS} - 1.03 \text{ ADL}$; RSD = 2.13%, $R^2 = 0.77$, $P < 0.0001$.

The OMS method was superior to chemical composition and to the *in vitro* rumen fluid method in predicting OMD of hays from natural pastures. Results from PCA suggested that it may be useful to group these hays according to its chemical composition to accurately predict OMD.

Key words: *in vitro* digestibility, *in vivo* digestibility, meadow hays, pepsin-cellulase

Introduction

Ruminants form a major component of livestock production in the Northeast region of Portugal. The climate is characterized by relatively rigorous winters and hot-dry summers. In this way, ruminants are mainly grazed and/or browsed on available natural pastures ("lameiros").

However, during summer, there is an acute shortage of feed and available forages are of very poor nutritive quality (i.e., low in crude protein and high in fibre), resulting in low feed digestibility and low voluntary intake. Hays are traditionally used as the main feed sources during the period of drought with scarce vegetative growth. An adequate nutrition is essential to exploit the genetic potential of livestock and several methods of feed supplementation for ruminants under such management system have been investigated. Evaluation of the nutritive value of hays is essential to provide a basis for development of adequate diets for ruminants.

The nutritional value of the fodder plants in general, and of hays in particular, can be estimated with adequate precision from *in vivo* digestibility. However, this requires high economic costs and long periods of time for each determination. The development of laboratory methods for predicting the nutritive value of ruminant feeds has been an active area of research for over 40 years. Methods using rumen fluid have widely and successfully been used to determine forage organic matter digestibility (Aerts et al 1977; Gasa et al 1989; Alexander and McGowan 1996). Due to the problems in standardizing *in vitro* techniques based on rumen fluid, much effort has been placed to develop enzymatic methods (Jones and Theodorou 2000). Methods based on fungal enzymes (*Trichoderma sp*) have resulted in more precise and repeatable predictions of organic matter digestibility of forages (Aufère and Michalet-Doreau 1988; Givens et al 1993a, b; Nousiainen et al 2003a, b).

The present work was designed to test the ability to predict the apparent organic matter (OM) digestibility (OMD) of hays from natural pastures from Northeast region of Portugal using chemical composition and laboratory digestibility measurements using enzymes or rumen fluid.

Materials and methods

Study area

The study area is located in the Northeast zone of Portugal (latitude 41° 49' N, longitude 6° 40' W), at an altitude higher than 700 m above sea level. The average annual temperature is 11.9°C, with an average minimum temperature of 0.9°C (January) and a maximum average temperature of 21°C (July). The relative humidity varies between 60 and 84% and the mean annual rainfall is 741 mm.

Normally, the soils are low in organic matter and of acid reaction. They have low cation exchange capacity and available phosphate and high or medium available K₂O (Martins and Coutinho 1988).

Hays

The botanical composition of hays varied within this region. Gramineae were dominant in all hays (60 to 80%), with *Agrostis castelana* L, *Festuca rubra* L, *Holcus lanatus* L, *Lolium perenne* L and *Bromus* spp as the main plant species. However, in some regions, plant species from Juncaceae and Cyperaceae were present at a proportion varying from 20 to 30%. The legume species represented a low proportion in all hays (lower than 10%).

Twenty one natural pastures from this region were randomly chosen. Forages were harvested at the soft dough stage of grasses and haymaking was processed by the producer.

Approximately 500 kg of hay were collected on each farm after baling. Bales were chosen at random and were taken to the Experimental facilities at the High School of Agriculture (Bragança, Portugal).

Determination of the in vivo apparent digestibility

Eight adult rams from the local breed Churro Galego Bragançano (live weight 63±1.2 kg) were used for the determination of the OMD by total collection of faeces. Before the beginning of the experiment the animals were shorn and given an anthelmintic drench (Ivomec, Portuguese Merial) and an intramuscular injection of fat soluble vitamins supplying 500000 IU vitamin A, 75000 vitamin D, and 50 IU vitamin E (Vitinject AD₃E, Vet Forte, Cipan). The animals were housed in metabolic cages allowing separate collection of faeces and urine in a well ventilated building.

The animals were randomly divided into two groups of 4 animals each. Digestibility was determined with 4 animals, in series of 2 hays chosen randomly. For each series (2 hays) the duration of the experimental period was about 12 days, 5 days for adaptation and 7 days for total collection of faeces and refusals. Once the digestibility of 8 hays were determined (4 series) animals were allowed a resting period of about 15 days in a conventional barn. Before and after each series, animals were weighed in the morning.

The hays were coarsely chopped (5 cm) to reduce wastage and selection during feeding, and were offered during the day with an allowance of 15-20% refusals. Animals also received a mineral-vitamin mixture representing about 1.5% of the daily dry matter (DM) intake and had free access to water.

Samples of the feeds offered were taken daily. After the experimental period (12 days), feed samples were bulked and dried at 65°C for at least 24 h to determine DM. Refusals and faeces were collected daily. Refusals were dried as described above for DM determination. After collection, faeces were carefully homogenised and about 20% (weight basis) were sampled and stored at -15°C. After the collection period (7 days), frozen faeces were bulked and dried as described above for DM determination. Bulked and dried samples of feeds, refusals and faeces were ground through a 1 mm screen for laboratory analysis.

Determination of in vitro digestibility

Three mature Churro Galego Bragançano sheep fitted with permanent rumen cannulae were used to collect rumen liquor for the in vitro incubations. Animals were fed, twice a day (8.00 h and 20.00 h), medium quality meadow hay from the Northeast region of Portugal (80 g CP/kgDM) at levels close to the maintenance feeding level. All the animals were kept in well-ventilated facilities and had free access to water and to mineral-vitamin blocks.

For the determination of the in vitro of OM digestibility (IVOMD) rumen liquor was collected 2 h after the morning feeding and pooled into a pre-warmed thermos flask flushed with CO₂. Before use in the laboratory, rumen fluid was strained and filtered through cheesecloth. The IVOMD was determined on dried and ground (1 mm) samples by the two-stage procedure of Tilley and Terry (1963) as modified by Marten and Barnes (1980). Each sample was incubated in duplicate (2 tubes per sample) and repeated in 2 runs carried out in different days. After incubation, residues were rinsed in cold water and were subjected to ash determination. Six standard hay samples of known in vivo digestibility were inserted in each run as references to measure variability among runs.

Pepsin-cellulase solubility

The solubility of OM by pepsin-cellulase (OMS) was determined on dried and ground (1 mm) samples using the procedures describe by Aufrère (1982). Samples (500 mg) were incubated with a pepsin (Sigma P7000) solution containing 2 g of pepsin per litre and HCl 1 N for 24 h at 40°C followed by the incubation with a buffer solution (pH 4.6) with 1 g cellulase from *Trichoderma viride* (cellulase

Onozuka R10, Medicine Department, Yakult Honsha Co, Ltd) per litre for 24 h at 40°C. After incubation, tubes were removed from the bath and the residues were subjected to ash determination. Each sample was incubated in duplicate (2 tubes per sample) and repeated in 2 runs carried out in different days. Two standard meadow hay samples were included in every incubation run to verify the activity of the enzyme and to follow standard laboratory procedures.

Chemical analysis

Standard methods as described in AOAC (1990) were used for determination of ash in feed, refusals, faeces and residues from in vitro incubation with rumen fluid and with pepsin-cellulase.

Feed samples were also analysed for crude protein (CP) and fibre fractions. The CP was assayed by the Kjeldahl method (AOAC 1990), modified by using a solution of boric acid (40 g/l) to receive free ammonia during distillation; a solution of 2 g/l of bromo-cresol green and 1 g/l of methyl red in ethanol as indicator and a standard acid solution (1N HCl) for titration. The neutral detergent fibre (NDF) was determined without sodium sulphite and amylase and was ash free (Van Soest et al 1991). Acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Robertson and Van Soest (1981).

Statistical analysis

All statistical analysis were performed using the statistical procedures of SAS (1990).

Data were subjected to correlation and regression analysis to study relationships between chemical composition, OMS, IVOMD and OMD. The simple and multiple regression equations were evaluated with the determination coefficient (R^2) adjusted for the degrees of freedom and the residual standard deviation (RSD). To estimate OMD of hays by multiple linear regression equations, several independent variables were considered according to a stepwise regression analysis. Equations were fitted by the least square method following a stepwise procedure with the 0.05 probability level for inclusion or deletion of variables.

Principal components analysis (PCA) was performed based on the correlation matrix of chemical composition and laboratory digestibility measurements.

Results

Chemical composition and digestibility of hays

The means, range of values, standard error (SE) and coefficient of variation (CV) of DM, chemical composition, IVOMD, OMS and OMD of the hays is shown in Table 1.

Table 1. Mean values, range of values, standard error (SE) and coefficient of variation (CV, %) for dry matter, chemical composition and digestibility measurements of hays (n=21)

Measurement	Mean	Range	SE	CV
Dry matter, g/kg	896	867 – 920	0.79	1.5
<i>Chemical composition, g/kg DM</i>				
Ash	66	42.9 – 78.1	0.64	13.3
Crude protein	73	55.7 – 84.7	0.62	11.3
Neutral detergent fibre	673	630 – 726	1.17	4.3
Acid detergent fibre	425	397 – 463	1.00	4.9
Acid detergent lignin	56	40.6 – 78.4	0.70	18.7
<i>Digestibility¹, g/kg OM</i>				
In vitro organic matter digestibility	378	313 – 418	0.37	7.7
Ppsin-cellulase solubility of organic matter	442	386 – 487	0.37	6.4
Apparent in vivo digestibility of organic matter	579	516 – 658	0.44	7.1

¹ IVOMD; OMS; OMD.

Most chemical components showed a wide range of values. The larger variation was observed for ADL content (CV=18.7%) and the lower variation was observed for DM content (CV=1.5%). The NDF fraction represented the greatest proportion of the samples, ranging between 630 and 726 g/kg DM. The CP content was lower than 85 g/kg DM.

The variation was similar between the methods used for the digestibility measurement (CV ranged from 6.4 to 7.7%). However, the values obtained from the method based on rumen fluid (IVOMD; 378 g/kg OM) were lower than those obtained by the enzymatic procedure (OMS; 442 g/kg OM). The apparent OMD varied between 516 and 658 g/kg OM.

Prediction of apparent digestibility from chemical composition and laboratory digestibility measurements

Prediction equations of OMD of hays by single regression analysis are presented on Table 2.

Table 2. Prediction of apparent in vivo organic matter digestibility of hays (n=21) from chemical composition and laboratory digestibility measurements¹

Variable ²	Intercept	SE ³	Coefficient	SE ³	RSD ⁴	R ²	P-value
CP	40.9	0.72	2.35	0.98	3.6	0.23	0.027
NDF	111	1.7	-0.79	0.26	3.4	0.29	0.007
ADF	105	1.5	-1.10	0.37	3.4	0.29	0.007
ADL	69	0.44	-1.85	0.78	3.6	0.23	0.029
IVOMD	26	0.95	0.86	0.25	3.3	0.38	0.003
OMS	11.4	0.98	1.06	0.22	2.8	0.52	<0.0001

¹all variables were expressed as percentage; ²CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; IVOMD, in vitro organic matter digestibility; OMS, pepsin-cellulase solubility of organic matter; ³SE, standard error; ⁴RSD, residual standard deviation

All the chemical parameters (CP and cell wall components) predicted OMD with low, and not acceptable, accuracy (R² varied between 0.23 and 0.29; P>0.05; RSD >3.4%). Significant negative regression coefficients were obtained with NDF (P<0.01), ADF (P<0.01) and ADL (P<0.05), while the regression coefficient with CP was positive (P<0.05). Chemical composition explained less than 30% of the variability in OMD observed for meadow hays. In contrast to chemical parameters, OMS explained 52% of OMD digestibility of hays and was the best single OMD predictor (RSD = 2.8%, R² = 0.52, P<0.001). The IVOMD led to a lower prediction accuracy (RSD = 3.3%; R² = 0.38, P<0.01).

When a multiple regression was performed between the OMD and the chemical composition or laboratory digestibility measurements, the variables included in the model were OMS and ADL as:

$$\text{OMD} = 2.15 + 0.94 \text{ OMS} - 0.83 \text{ ADL}; \text{RSD} = 2.75\%, \text{R}^2 = 0.54, \text{P} < 0.0001$$

As expected, the ADL estimated coefficient had a negative sign and the OMS estimated coefficient had a positive sign. However, this bivariate model improved prediction accuracy only slightly compared to single regression equations presented on Table 2.

Principal components analysis

In order to explain differences of digestibility of meadow hays and to create new independent variables with better capacity to predict OMD of meadow hays, PCA was conducted on chemical composition and laboratory digestibility measurements. The principal component 1 (PC1), a new independent variable created by PCA, was significantly correlated with the OMD of the meadow hays (r=0.66; P<0.01). However, it explained less of the OMD variation than OMS (Table 2).

Figure 1 shows plots of the chemical composition and laboratory digestibility measurements on the two first components (representing 67.4% of the variability).

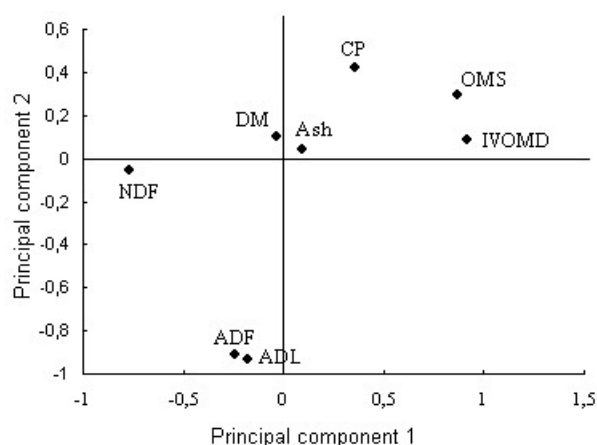


Figure 1. Projections of the chemical composition and laboratory digestibility measurements of the hays in the plane defined by the two first principal components. DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; IVOMD, in vitro organic matter digestibility; OMS, pepsin-cellulase solubility of organic matter

All the digestibility measurements (IVOMD and OMS) had similar proportions in the PC1 having a positive relation with it. The second principal component (PC2) is characterized by two measurements, ADF and ADL, having also similar proportions in the PC2. In this loading plot, the PC1 exhibit the opposition between NDF and the digestibility measurements and the PC2 is mainly explained by the opposite of the ADF and ADL contents and the CP content.

Discussion

Chemical composition and digestibility

A large variation in chemical composition between hays was observed confirming previous results reported by Ferreira et al (1981) and Rodrigues et al (2007) with meadow hays from the Northeast of Portugal. Hays from native pastures contain many natural species which have different biological growth rates and different composition. Great variation in chemical composition was also observed by Lopez et al (1991) with hays from 10 permanent mountain meadows in North-western Spain and by Andrighetto et al (1992) with hays from native alpine mountain pastures (n=28).

On average, the sum of ash, CP and NDF accounted for 812 g/kg DM. Since ether extract of meadow hays from this region is lower than 10 g/kg DM (Guedes and Dias-da-Silva 2005) the non-structural carbohydrates may represent approximately 180 g/kg DM.

The CP level ranged between 55.7 and 84.7 g/kg DM and accords with previous results (Ferreira et al 1981; Rodrigues et al 2007). Our results showed that ADL was the most variable cell wall component which also agrees with findings reported by Rodrigues et al (2007) (CV=16.9%). Ash content varied between 43.0 to 78.1 g/kg DM and was probably affected by soil/dust contamination of the hays.

The OMD values observed in our study (579 ± 0.41 g/kg OM) were similar to those obtained by Ferreira et al (1981) with 19 meadow hays (OMD= 546 ± 0.52 g/kg OM) but were higher than those reported by Fonseca et al (1998) in two meadow hays (OMD=496 and 522 g/kg OM).

It was observed that the OMS method gave higher values than the rumen liquor method, regardless of hay digestibility, indicating that the OMS and IVOMD solubilised somewhat different fractions of the hays.

Predicting apparent digestibility from chemical composition and laboratory digestibility measurements

These results confirm previous findings stating that chemical analysis alone will be of limited value in predicting the nutritive value of hays (Andrighetto et al 1992; Alexander and McGowan 1996). In agreement with our study, Andrighetto et al (1992) found that chemical parameters did not predict OMD of hays from native mountain pastures. This can be attributed to the heterogeneous material used due to the effects of the different geographical location, plant species, soil type and conservation process of the hays. Nouasiainen et al (2003a) observed that chemical components were particularly poor as predictors of OMD of primary growth and regrowth silages produced in uniform conditions at the same farm. If the accuracy of chemical composition for predicting OMD is not acceptable using such a homogeneous material, it is obviously not suitable in evaluating more heterogeneous samples as the hays from natural pastures.

Van Soest et al (1978) suggested that NDF and ADF failed to satisfactorily predict the digestibility of forages and Van Soest (1994) presented experimental data from a large number of samples which seriously question the reliability of regression equations using single fibre measurements, NDF and ADF, to predict forage digestibility. This is due to the differing environmental factors promoting lignification as opposed to cell wall content (Van Soest et al 1978). Therefore, the negative association between cell wall and digestibility would be dependent on lignification of cell wall. In the present study, although ADL content varied greatly (CV = 18.7%), we did not find any correlation between OMD and the ratio of ADL/NDF ($r = -0.35$; $P > 0.05$) and we found no strong correlation between ADL/ADF ratio and the OMD ($r = -0.43$; $P < 0.05$).

The best equation for predicting OMD was obtained with the OMS method. This is in agreement with the results of Andrighetto et al (1992) and Narasimhalu (1984) with hays, Givens et al (1990, 1993a) and Nouisainen et al (2003a, b) with grass silages and Givens et al (1993b) with fresh herbage. Givens et al (1989) with grass silages and Givens et al (1993b) working with spring-grown herbage from England and Wales obtained prediction errors (RSD) for pepsin-cellulase solubility comparable with those found in our work (3.10 and 3.23%, respectively) and the regression equations were also similar to ours. Andrighetto et al (1992) applied the same enzymatic method to hays from native mountain forages (n=28) to estimate OMD and found a predicting error of 3.43%. Similarly, Narasimhalu (1984) estimated the OMD of 18 grass hays using an enzymatic method with cellulose from Onazuka with an accuracy similar to that found in our study (RSD=2.71% and $R^2=0.58$).

The best multiple regression equation determined by stepwise analysis was a bivariate model with the OMS and the ADL. However, this equation, although significant, did not provide sufficient reduction in predicting errors to justify the use of bivariate relationships. Using 18 grass hays, Narasimhalu (1984) also found that in vivo DM digestibility was accurately estimated by multiple regression from the ADL content and the pepsin-cellulase method ($R^2=0.69$). However, the single regression with the pepsin-cellulase methods obtained by these authors explained only 25% of the total variation of estimated DMD and the variation accounted for by the regression increased to 69% when ADL content was included as the second independent variable in the prediction equation. The ADL values found by these

authors showed a higher variation (ranged from 23 to 67 g/kg DM) than the ADL found in our work which may explain their results.

It seems clear that the material used was non-uniform, affecting the relationship between the values of in vivo digestibility and the analytical results. However, both equations (single and multiple regressions) indicate that OMS was able to predict OMD of these hays, in comparison with the in vitro technique using ruminal fluid. Although the usefulness of the rumen fluid in vitro incubation has been presented by several authors (Clark and Beard 1977; McLeod and Minson 1978; Pace et al 1984; Gasa et al 1989) the comparison in prediction precision between rumen fluid in vitro incubation and enzymatic techniques have produced variable results (Givens et al 1989, 1990) and there are some inherent problems in the use of these techniques (Moss and Givens 1990) related mainly to the variability of the rumen fluid. Furthermore, the OMS method is the simplest in vitro technique from the operative point of view.

Principal components analysis

Figure 2 displays the projection of the chemical composition and laboratory digestibility measurements of the meadow hays in the first two principal components (PC1 and PC2).

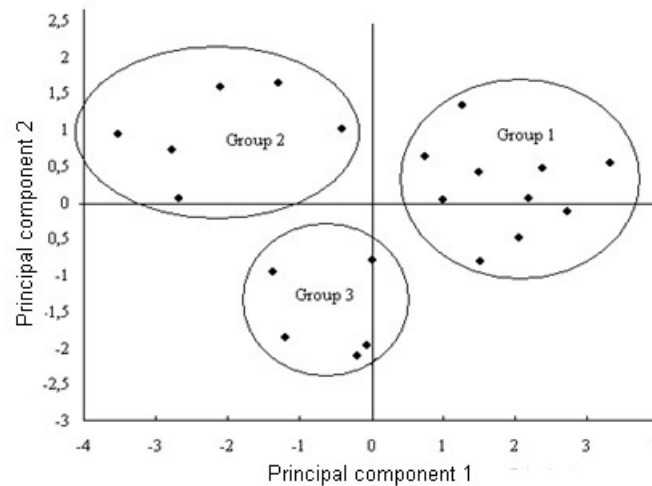


Figure 2. Projection of the chemical composition and laboratory digestibility data of the hays studied in the plane defined by the two principal components

Hays located in the left side of the figure, where cell wall constituents lay, are those dominated by a negative relation between laboratory digestibility measurements and cell wall. The hays dominated by a positive relation between the CP contents and the laboratory digestibility measurements lie on the right side of the figure, where the CP contents lay.

The projection of the feedstuffs on the plan of the first two components highlighted differences between feedstuffs. Hays with high ADL content were close to the cell components (NDF, ADF and ADL) and opposed to OM digestibility measurements (OMS and IVOMD). Graphically, this shows that digestibility and cell wall constituents are negatively correlated when hays presented higher correlation between cell wall components. On the contrary, when the cell wall constituents were lower, OM digestibility measurements were positively correlated with CP content. Based on Figure 2, three separate groups of hays can be observed. Differences between these groups may be partly attributed to the variation in chemical composition, in particular, the NDF and CP contents. Hays in group 1 presented a low concentration of all cell wall components and a high CP concentration. Hays from this group also presented higher values of IVOMD and OMS, suggesting that they may be of higher quality compared to hays in groups 2 and 3. In fact, mean OMD of hays from group 1 was higher (613 g/kg OM) than that observed for hays from group 2 (550 g/kg OM) and from group 3 (558 g/kg OM).

A multiple regression was established between the OMD and the chemical composition or laboratory digestibility measurements for hays from group 1 (n=10) and the variables included in the model were also OMS and ADL. However, the accuracy of the prediction increased compared to that obtained using all the hays. The multiple regression was:

$$\text{OMD} = 4.13 + 0.85 \text{ OMS} - 1.03 \text{ ADL}; \text{RSD} = 2.13\%, \text{R}^2 = 0.77, \text{P} < 0.0001$$

These data suggest that it will be useful to group the hays according to its chemical composition (mainly NDF and CP) in order to increase the accuracy of OMD estimation.

Conclusions

- The OMS method was superior to chemical composition and to the in vitro rumen fluid method in predicting OMD of hays from natural pastures.
- A bivariate regression equation including OMS and ADL content to predict OMD of these hays was found (RSD = 2.75%; $R^2 = 0.54$)
- After PCA analysis, it was evident the usefulness of grouping the hays according to its chemical composition (mainly NDF and CP content) in order to increase the accuracy of OMD prediction.

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Received 17 May 2008; Accepted 29 May 2008; Published 5 August 2008

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