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DAKAR - HANN

"IN SITU" DEGRADATION OF RESSOURCES  
FOR ANIMAL FEEDING :  
EV-ALUATION OF THE NYLON BAG TECHNIQUE

By

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## S U M M A R Y

The nylon bag method is a mean for evaluation of nutrients availability in the rumen.

Various factors of variation limiting the accuracy of the technique are checked.

Five grams of dried, and ground rice straw, in a nylon bag, are incubated in the rumen for 24, 48 and 72 hours.

Removed bags are washed and oven dried at 80°C. The percentages of dry matter and cell wall disappearance represents respectively dry matter and NDF degradability in the rumen.

Pore size of nylon material had a significant ( $P \leq 0,05$ ) influence on dry matter and cell wall degradability. This influence was not significant ( $P \leq 0,05$ ) at 72 hours of incubation.

The test limited the contribution of bags to the gastric motility. Its influence on nutrient degradability has been negative.

Results with cattle and sheep have not been significantly ( $P \leq 0,05$ ) different.

The bag size had no effect on nutrient degradability.

The test has been an important source of variation.

A good quality forage with energetic and nitrogen supplement is required.

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Degradability of rice straw nutrients have been significantly higher with the diet based on peanut haulms than with the composed diet based on sorghum, Peanut cake and peanut shell.

The between animals and trials differences recorded have been significant.

Animal have not to be less than three animal a single bag can be incubated per incubation time, with one repetition trial per sample to have a reliable mean.

The effect of pepsine Post rumen incubation treatment have not been significant.

Cell wall and dry matter degradability of rice straw was good.

48 hours incubation degradability of rice straw and in vivo results have not been different.

Keywords : nylon bag technique ; variation factors ; in "situ" degradation of nutrients ; dry matter and cell wall rumen degradability of rice straw.

## I INTRODUCTION

The digestion study is an important step in the process of nutritive value evaluation of feedstuffs.

Chemical analysis determines the quantity of nutrients in the feedstuffs, but doesn't give informations about their availability.

In vitro techniques are easier and less expensive than in vivo and in situ methods ; but they don't really reflect the digestive utilisation of feedstuffs.

The nylon bag method is a technique for measurement of the kinetic of in situ nutrients degradation in the rumen.

It gives informations about potentially degradable fraction of nutrients and it's degradation rate.

This method can evaluate the availability of nutrients and complete bromatological analysis.

Since the initial silk bag of Quin et al in 1938, many methodological studies about nylon bag have been carried out (RODRIGUEZ 1968, CHENOST 1970, MEHREZ et al. 1977, KEMPTON 1980, UDEN and VAN SOEST 1984, SAUVANT et al. 1985). Many research teams have improved the accuracy of the technique.

Efforts of standardization and comparison with related methods are continuing.

However, a literature review shows a great variety of material used and experimental procedure from a laboratory to another (see table 1).

The aim of this methodological study is to adjust the nylon bag technique in our laboratory and to check different factors of variation.

Table 1 : Bibliographical variations of experimental conditions

Authors	Pore size $\mu$	Sample size g	Screen mm	Bag size cm	Sample/ bag size mg/cm <sup>2</sup>
VAN KEUREN 1962	-	10	- -	5,1 x 11,4	86,0
PLAYNE 1978		3		6 x 6	41,7
PLAYNE 1978		6		6 x 12	41,7
PLAYNE 1978		9		6 x 18	41,7
FIGROID 1972	-	10		10,2 x 17,8	27,5
MEHREZ 1977		5		17, x 9	16,3
BAILEY 1962		5		10,2 x 15,2	16,1
DEMARQUILLY 1969		3	-	15 x 7	14,3
AERTS 1976		3		15 x 8	12,5
Mc MANUS 1972		2		15 x 15	4,4
ISTASSE et al. 1981	50	2		6 x 7	23,3
ORSKOV 1980	12	3	2,5 à 3	14 x 9	11,9
LOERCH 1983	20 à 70	*		8 x 14	-
CHENOST et coll. 1970	<50	3		15 x 7	14,2
MASTER et coll. 1983	43	2	-	3 x 21	15,8
OKEKE et coll. 1983	10	5		9 x 16	17,3
KEMPTON 1981		3 à 5	1	15 x 8	12,5 à 20,8
MEHREZ et ORSKOV 1977	-	4 8 5		20 x 9	11,1 à 13,8
DEMARQUILLY et JARRIGE 1981	50	3	1	15 x 7	14,2
ORSKOV 1984	20 à 40	3 à 5	2,5 à 3	14 x 9	11,9 à 19,8
De FARIA 1984	35	2		12,5 x 9	17,7
UDEN et al. 1984	37		>2	5 x 12	6 à 7
STERN et al. 1984	52 ± 16	0,5	1	6 x 10	4,1
ANDERSON et al. 1981	150	0,5	"	6 x 15	2,7
VAN DER AAR 1982	20 à 70	*		8 x 14	
VAN SOEST 1982	30				

\* Quantité équivalente à 250 mg de protéines brutes.

## II - MATERIAL AND METHOD

### 2.1 - The material

#### 2.1.1 - The nylon bag

A nylon material with regular pore size = 30 microns (1) have been compared to an irregular pore size nylon material varying from 10 to 80 microns (2).

Two bag sizes have been tested 10 x 15 cm and 10 x 20 cm.

#### 2.1.2 - The animals

Three cattle and three sheep, holding fistula with 75 and 60 mm of diameter have been used.

Mortality of sheep caused by fistula parasitisme by f'ly larva was important. Exploited results concerned only one a sheep.

#### 2.1.3 - The sample

All measurements have been done with a test rice straw harvested in 1985 in Senegal river delta. This crop residue was oven dried at 80°C and ground to pass 1 mm screen.

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(1) Beck Michel et Simons - France.

(2) F100 Tripette et Renaud - France.

#### 2.1.4 - Animal diet

Two diet was tested :

- peanut haulms, harvested in 1984 in Nioro aeria was offered at libitum to sheep and cattle.  
The intake level reached 126 g dry matter per kilogram metabolic body weight for cattle.  
Approximate nutritive value of peanut is : 0,45 forage unit  
60 g digestible nitrogen, 10.1 g calcium and 1.2 phosphorus per kilogram of dry matter ;
- a composed diet with 68 p.100 of peanut shell, 27 per cent of sorghum, 5 percent peanut cake, was offered at libitum,  
Intake was 118 g dry matter per kilogram metabolic body weight and nutritive value 0,33forage unit, 53digestible nitrogen, 7 g calcium and 1,6 g phosphorus.  
A Mineral block was available in free choice.

#### 2.1.5 - The lest

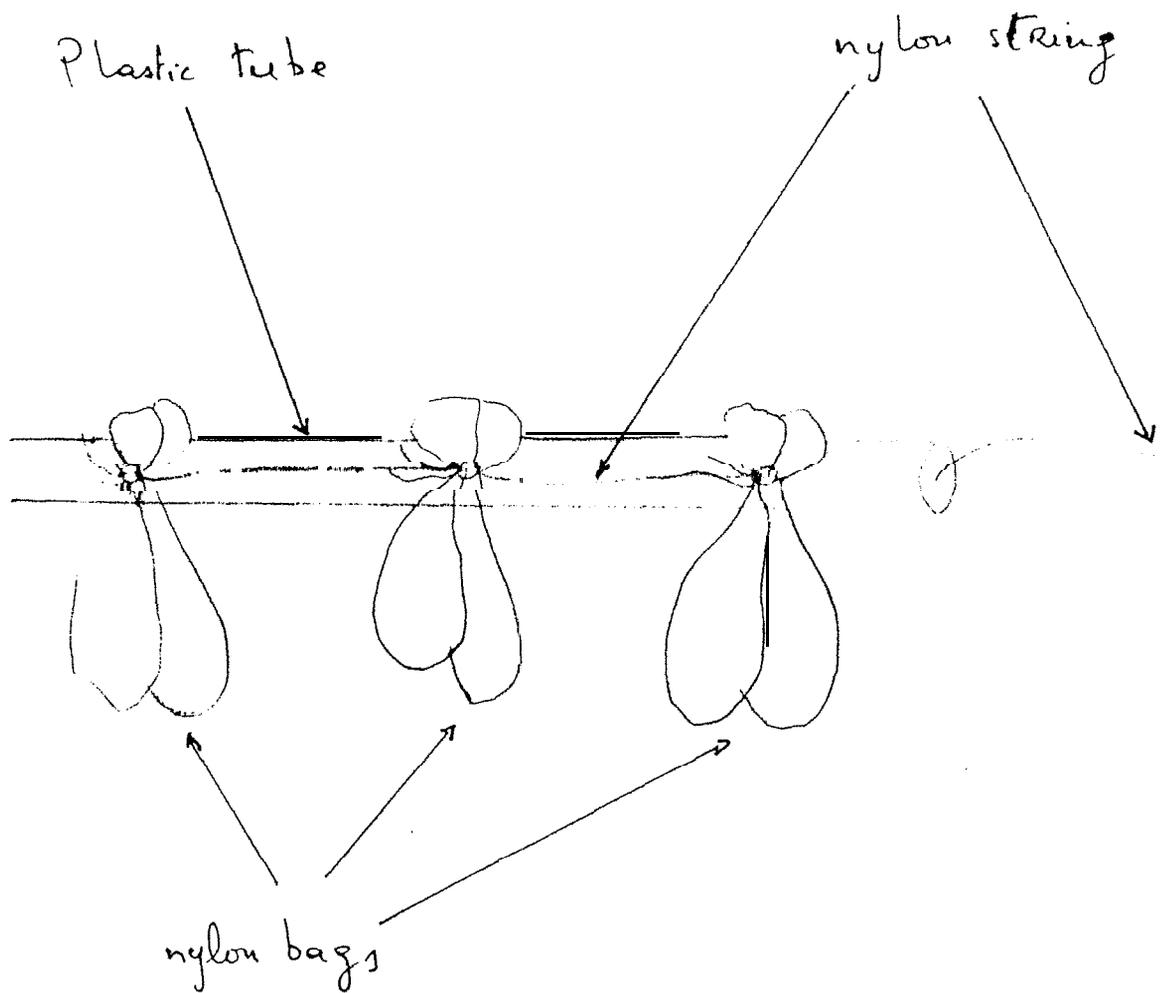
It's a lead sphere covered with plastic. The approximate weight is 100 g for shecp and 350 g for cattle.

The lest prevents maintenance of bags in the rumen content surface and keep them deeply mixed with the digesta.

Bags are attached to a plastic tube according to orskov's model (see fig. 1) in order to make easy the removal of the bag from the rumen through the fistula.

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Figure 1: Attachment system of bags. (Orskov 1984)



## 2.2 - Experimental procedure

### 2.2.1 - The incubation

Five grams of dried ground sample are introduced in a nylon bag. The bag is heat sealed and introduced in the rumen through the fistula 30 minutes after morning feeding.

Double nylon bags are removed 24, 48 and 72 hours later.

They are massed under running water at room temperature until the outflow water is clear. Bags are oven dried.

The percentage of **dry** matter loss represents **dry** matter degradability **i n** the rumen.

A double bag **with non** incubated sample, sealed and **washed** represents the **zero point**.

### 2.2.2 - Chemical analysis

Cell wall (NDF) fraction is determined **i n t h e** sample and the **residue** after incubation.

pH, ammonia level and total volatile fatty acids are measured in the rumen fluid for characterisation of compared diets.

### III -- RESULTS AND DISCUSSIONS

Different variations factors have been studied with about twenty methodological trials.

Variations concern material and experimental procedure.

#### 3.1 Material variations

##### 3.1.1-- Pore size of nylon material

In fact, the degradability of nutrients is assimilated to the percentage of them escaping the nylon bag. The pore size of nylon material is then an important parameter.

Usually it varies from 10 (OKEKE et al. 1983) to 150 microns (ANDERSON et al.) (see table 1).

Compared pore size of 30 microns and 10 to 80 microns gave significantly ( $P \leq 0,01$ ) different results (see table 2 and 3) at 24 hours of incubation.

Differences between the two nylon material type at 48 hours incubation was significant ( $P \leq 0,025$ ) for dry matter but not for cell wall degradability ( $P \leq 0,05$ ).

At 72 hours of incubation no difference have been significant ( $P \leq 0,05$ ).

Variations are important during the first moments of incubation, when occurs degradation of protein. The standardization of nylon material is then a necessity.

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Table 2 : Nylon material effect on dry matter and NDF degradability of rice straw

Incubation time h	nylon material	dry matter degradability p.100			NDF degradability p.100		
		Mean	Standard deviation	Number of trials	Mean	Standard deviation	Number of trials
24	H <sub>1</sub>	29.4	3	15	25.7	3.0	12
	H <sub>2</sub>	37.3	5.8	20	32.6	5.7	6
48	H <sub>1</sub>	49.5	9.4	7	49.7	10.9	6
	H <sub>2</sub>	56.8	3.3	17	53.3	5.9	18
72	H <sub>1</sub>	62.2	6.2	3	61.9	4.14	3
	H <sub>2</sub>	63.2	4.3	12	62.8	7.0	18

H<sub>1</sub> = Regular pore size = 30 microns  
Nylon material from Beck Michel et Simon - France

H<sub>2</sub> = Irregular pore size = 10 - 80 microns  
Nylon material F100 from Iripette et Renaud - France.

**Table 3** : Influence of porc size on dry matter and NDF degradability of rice straw analysis of variance

Incubation\ time	F		F	
	Dry matter degradability		NDF degradability	
24 heures	$F_{34}^1 = 22,14^{****}$		$F_{17}^1 = 9,86^{****}$	
48 heures	$F_{23}^1 = 7,88^{**}$		$F_{23}^1 = 0,92$	NS
72 heures	$F_{22}^1 = 3,10$	NS	$F_{20}^1 = 0,04$	NS

NS = non significant

\* significant at  $P \leq 5\%$

\*\* significant at  $P \leq 2,5\%$

\*\*\* significant at  $P \leq 1\%$

\*\*\*\* significant et  $P \leq 1\%$ .

Table 4 : Sample / bag size effect on dry matter and NDF degradability of rice straw

Incubation time (h)	Sample/bag size mg/cm <sup>2</sup>	Dry matter digestibility P.100			NDF digestibility P.100		
		mean	Standard deviation	Number of trials	mean	Standard deviation	Number of trials
24 h	16.6	57.5	5.8	20	28.5	5.7	14
	12.5	34.9	2.5	7	22.9	2.6	4
48 h	16.6	56.8	3.6	17	50.2	3.6	7
	12.5	53.6	4.5	10	47.6	5.5	10
72 h	16.6	63.21	4.2	12	62.8	7.8	10
	12.5	57.8	5.5	6	57.6	8.6	3

Table 5 : Sample / bag size effect on dry matter and NDF digestibility of rice straw  
analysis of variance

Incubation time (h)	F		F	
	Dry matter		NDF	
24	$F_{26}^1 = 1.05$	NS	$F_{17}^1 = 3.63$	NS
48	$F_{26}^1 = 4.08$	NS	$F_{16}^1 = 0.8$	NS
72	$F_{17}^1 = 4.84^*$		$F_{20}^1 = 1.41$	NS

Recent publications give optimum pore size of 20 to 50 microns (CHENOST et al. 1970, VAN SOEST 1982, HASTER et al. 1983, ORSKOV 1984, De FARIA 1984, UDEN et al. 1984, STERN et al. 1984, SAUVANT et al. 1985, MICHALET DOREAU unpublished data).

Reproducibility of nylon bag method is better with regular pore size.

### 3.1.2 - Sample / bag surface ratio

The sample weight per unit of bag surface presents an important bibliographical variations (see table 1).

The mean ratio is 19,5 mg/cm<sup>2</sup> with extreme values from 86 (VAN KENREN 1962) to 2,7 mg/cm<sup>2</sup> (ANDERSON et al. 1981).

Comparative study between 16,6 and 12,5 mg/cm<sup>2</sup> has not shown significant differences ( $P \leq 0,05$ ) (see table 4 and 5).

Influence of bag size on degradability is not important.

The sample weight depends on analysis to be done in the residu. It has to move freely and participate to gastric motility within the bag.

### 3.1.3 The lest

Rumen content is non homogeneous.

The bag location in depth or surface and the degree of his participation to gastric movements, have an influence on the in "situ" degradation of nutrients.

Metallic sphere, isolated with plastic makes the bag apparatus heavy and deeply located in the digesta.

Table 6 : Influence of lost or dry matter and NDF degradability of rice straw

Incubation (h)	bag	Dry matter digestibility p.100			NDF digestibility p.100		
		Mean	Standard deviation	Number of trials	Mean	Standard deviation	Number of trials
24 h	With lost	34.9	2.5	7	23.1	2.0	6
	Without lost	39.6	3.7	12	29.9	3.3	12
48 h	With lost	53.6	4.5	10	47.6	5.5	10
	Without lost	57.4	2.6	10	53.1	6.4	11
72 h	With lost	57.8	6.5	6	57.6	6.6	3
	Without lost	61.4	7.0	17	62.2	5.8	8

**Table 7 : Influence of lests on dry matter and NDF degradability of rice straw**  
**Analysis of variance**

Incubation time (h)	F		F	
	Dry matter		NDF	
24	$F_{16}^1 = 8.36^{***}$		$F_{17}^1 = 19.57^{***}$	
48	$F_{19}^1 = 5.17^*$		$F_{20}^1 = 4.41^{**}$	
72	$F_{22}^1 = 1.26$	NS	$F_{10}^1 = 1.26$	NS

This procedure is not used by all research teams.

DEMARQUILLY 1981, BERGER et al. 1981, ISTASSE et al. 1981, UDEN et al. 1984, propose a test weight of 0,5 to 2,5 kg for cattle. KEMPTON 1980, PRESTON and LENG 1984, do not use any test. According to RODRIGUEZ 1968 the bag location effect on nutrients degradability is of minor importance.

Our comparative studies reveal a significant ( $P \leq 0,01$ ) superiority of degradability with non lested bag at 24 and 48 hours.

At 72 hours incubation time differences between lested and non lested bags are not significant ( $P \leq 0,05$ ) (see table 6 and 7).

Lest can be a limiting factor to the motility of the bag within the rumen and then depress the degradability of nutrient at the beginning of incubation.

The nylon string, holding the bag apparatus must be large enough to allow free movement of bags.

#### 3.1.4 - Animal specie

With sheep, our repetition number is limited (see table 8). However no significant ( $P \leq 0,05$ ) differences have been found between sheep and cattle (see table 8 and 9).

We are in agreement with observations of ORSKOV 1980, KEMPTON 1980 and UDEN and VAN SOEST 1984.

Sheep are easier to manage but fistula maintenance is more difficult for sheep than for cattle in tropical aeries and more bags can be incubated in cattle.

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Table 9 : Animal specie effect analysis of variance between sheep and cattle

Incubation time (h)	F		F	
	Dry matter		NDF	
24	$F_{9}^{1} = 3.36$	NS	$F_{9}^{1} = 0.93$	NS
48	$F_{23}^{1} = 0.35$	NS	$F_{21}^{1} = 0.1$	NS
72	$F_{25}^{1} = 3.56$	NS	$F_{23}^{1} = 3.81$	NS

Table 3 : Animal specie effect on dry matter and NDF degradability of rice straw

Incubation time (h)	Specie	Dry matter degradability p.100			NDF degradability p.100		
		Mean	Standard deviation	Number of trials	Mean	Standard deviation	Number of trials
24 h	Cattle	43.2	4.4	6	28.5	5.5	14
	Sheep	47.6	2.6	4	32.2	11.0	6
48 h	Cattle	55.5	4.9	19	50.6	4.0	17
	Sheep	57.6	10.9	5	52.1	14.4	5
72 h	Cattle	64.6	6.5	23	61.6	7.1	21
	Sheep	72.1	0.8	3	70.0	1.6	3

### 3.1.5 - Animal diet

Animal diet is an important variation factor in nylon bag method.

According to diet composition, rumen microflora can be specialized in cellulose (forage based diet) or starch degradation (diet with high level of cereals).

Biochemical characteristics of rumen fluid with the two tested diets are described in tables 10 and 11 and in figures 2 and 3.

Mean value of pH is normal in the morning. Ammonia and VFA rumen fluid concentrations are low.

Particularly the ammonia level is below the minimum of 5 to 8 mg/100 ml indicated by HARRISON and Mc ALLAN 1978, Mc MENNIHAN 1981, PRESTON and LENG 1985. It is far from the optimum level of ammonia of 20 mg/100 ml indicated by ORSKOV 1977.

However, the diet based on peanut haulms gave superior results compared to composed diet, because of the high level of sorghum which depressed cellulose degradation.

Offered with energetic and nitrogen supplement based on peanut cake, peanut haulm should be an excellent experimental diet.

However the nutritive value of this crop residue varies a lot according to the percentage of leaf attached to stems and their degree of lignification whose influence on nitrogen and energy availability is negative (CALVET, 1971).

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Table 10 : Diet based on pennut haulm biochemical characteristics of rumen fluid

Hour	pH			NH <sub>3</sub> mg/100 ml			VFA mEq/l		
	Number of trials	Mean	Standard deviation	Number of trials	Mean	Standard deviation	Number of trials	Mean	Standard deviation
8 h	36	7.15	0.20	30	2.46	0.27	30	19.8	4.7
10 h	37	6.02	0.29	30	2.95	0.47	31	19.8	3.1
12 h	37	6.69	0.27	31	2.94	0.58	31	21.2	4.9
14 h	35	6.66	0.32	29	2.66	0.48	29	21	0.8

Table 11 : Composed diet : Biochemical characteristics of rumen fluid

Characteristics Time of sampling	NH <sub>3</sub> mg/100 ml			VFA mEq/l			pH		
	Mean	Standard deviation	Number of trials	Mean	Standard deviation	Number of trials	Mean	Standard deviation	Number of trials
6 h	2.40	0.77	6	18.15	7.73	6	6.90	0.32	8
10 h	2.42	0.41	6	17.83	4.40	6	6.72	0.22	8
12 h	2.98	0.73	7	14.8	5.28	7	6.42	0.18	7
15 h 30	1.82	0.70	8	21.14	7.73	8	6.31	0.37	8
17 h 30	2.04	0.76	8	26.42	8.15	7	6.24	0.29	8

Figure 2 : Diet based on peanut haulm biochemical characteristics of rumen fluid

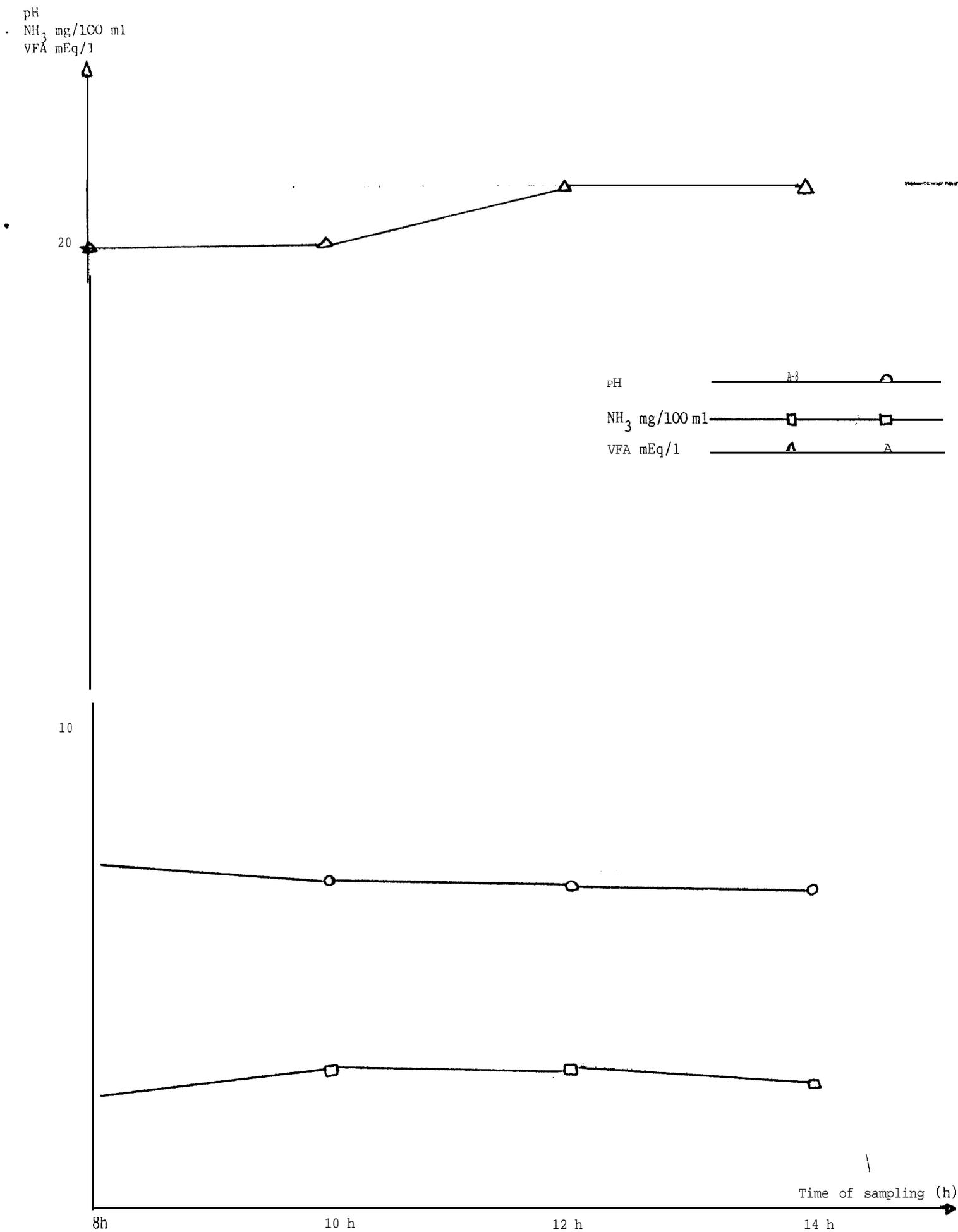


Figure 3 : Composed diet biochemical characteristics of rumen fluid

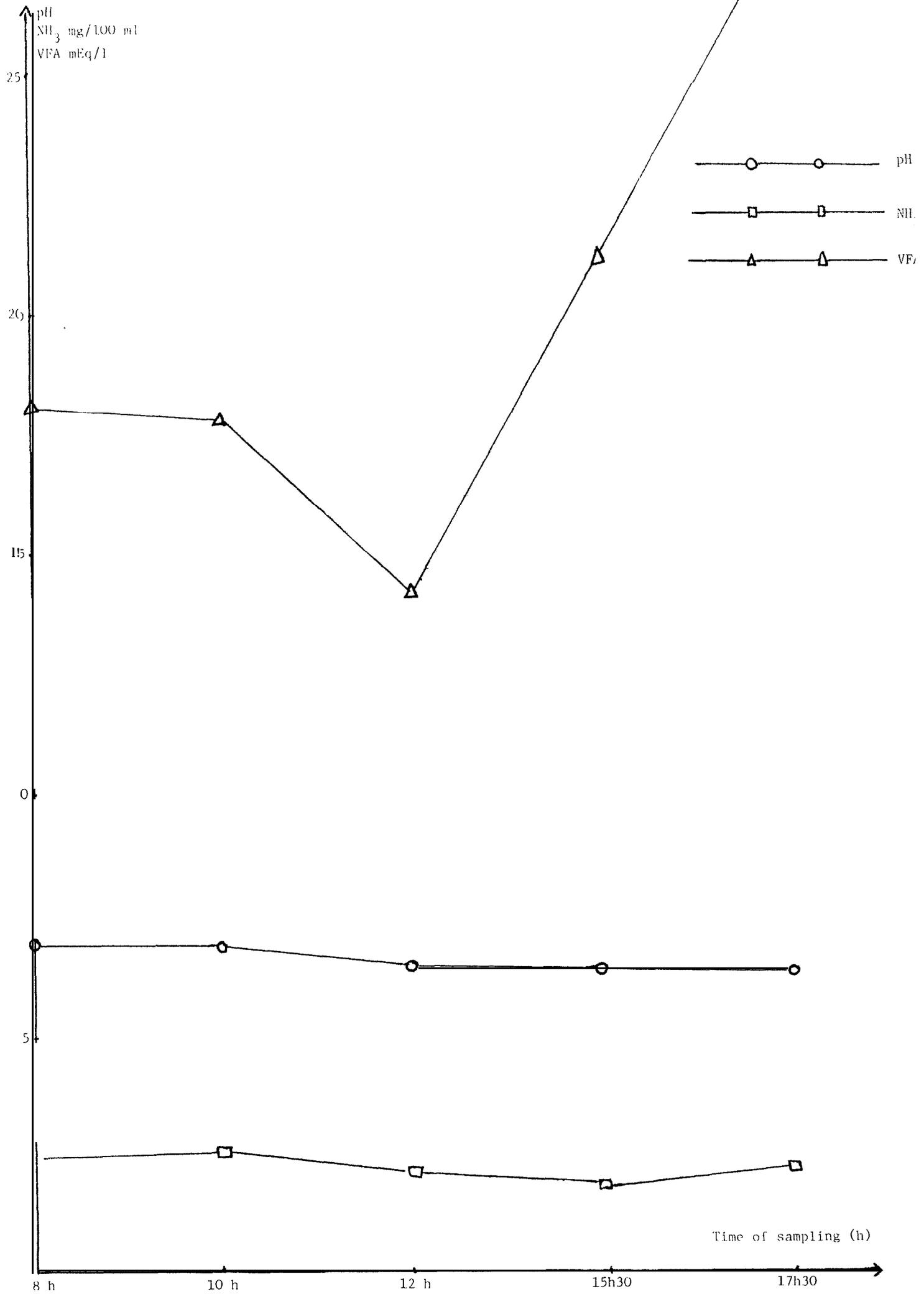


Table 12 : Influence of the diet on dry matter degradability of rice straw

Diet	Incubation time (h)	Dry matter degradability p.100		
		Mean	Standard deviation	Number of trials
Peanut hays	24	34.0	2.4	7
	48	54.8	3.8	3
	72	62.1	4.6	3
Composed diet	24	30.3	1.6	10
	48	34.3	1.4	10
	72	45.8	4.6	10

### 3.2 - Experimental procedure's variations

#### 3.2.1 - The number of repetition per sample

The rowett research institute's team have made detailed studies on this subject (ORSKOV, 1979).

According to Orskov's team the between animal variations is most important followed by the between trial and bag differences with respective variance of 6.2, 4.9 and 3.3 per cent.

An adequate repetition number, combining animal bag and trial number per sample is requested in order to have reliable mean value.

##### 3.2.1.1 Animal number

With F100 nylon material of variable pore size 10 - 80 microns, the between animal differences are irregular but often significant (see table 13).

For beck Michel & Simon nylon material with regular pore size the between animal differences are not significant ( $P \leq 0.05$ ).

An adequate non variable animal diet, with regular pore nylon material can reduce the between animal variations.

Animals should not be less than three.

##### 3.2.1.2 - The number of bag

On 127 double measurements of cell wall degradability and 131 double measurements of dry matter degradability, the mean between bag standard deviation was 1,59 with variations from 1 to 2 points.

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Table 13 : Between animal differences. Analysis of variance

Incubation time (hours)	Experimental conditions	Pore size = 30 $\mu$ bag size = 10 x 20 cm with leat diet = peanut haulm		Pore size = 10 - 80 $\mu$ bag size = 10 x 20 cm with leat diet = peanut haulm		Pore size = 10 - 80 $\mu$ bag size = 10 x 15 cm without leat diet = pennut haulm		Pore size = 10 - 80 $\mu$ bag size = 10 x 15 cm with leat diat = peanut haulm		Pore size = 10 - 80 $\mu$ bag size = 10 x 15 cm without leat with pepsine diat = peanut haulm	
		Measurements									
24 h	DDM	$F_{13}^1 = 0.00$	NS	$F_{20}^2 = 12.54^{***}$		$F_{17}^2 = 0.82$	NS	$F_{10}^2 = 96.14^{****}$		$F_{11}^2 = 13.92^{***}$	
	NDFD	$F_{11}^1 = 0.61$	NS	$F_{13}^1 = 0.01$	NS	$F_{11}^2 = 0.77$	NS	$F_{15}^1 = 3.53$	NS	$F_{11}^2 = 14.7^{***}$	
48 h	DDM	$F_{16}^1 = 3.50$	NS	$F_{16}^1 = 3.19$	NS	$F_{11}^2 = 10.10^{***}$		$F_{10}^1 = 7.25^{***}$		$F_{10}^2 = 17.74^{***}$	
	NDFD	$F_{15}^1 = 0.71$	NS	$F_{17}^1 = 6.41^{**}$		$F_{10}^2 = 0.11$	NS	$F_{9}^1 = 7.23$		$F_{10}^2 = 21.26^{****}$	
72 h	DDM			$F_{18}^1 = 1.56$	NS	$F_{16}^2 = 12.74^{****}$				$F_{10}^2 = 4.30$	NS
	NDFD			$F_{17}^1 = 1.82$	NS	$F_{20}^2 = 0.16$	NS			$F_{9}^2 = 3.71$	NS

DDM = Dry matter degradability

NDF = NDF degradability.

Table 14 : Between trial differences. Analysis of variance

Experimental conditions	Measurements	Pore size = 30 $\mu$ bag size = 10 x 20 cm with leest diet = peanut haulm	Pore size = 10 - 80 $\mu$ bag size = 10 x 20 cm with leest diet = peanut haulm	Pore size = 10 - 80 $\mu$ bag size = 10 x 15 cm without leest diet = peanut haulm	Pore size = 10 - 80 $\mu$ bag size = 10 x 15 cm with leest diet = peanut haulm	Pore size = 10 - 80 $\mu$ bag size = 10 x 15 cm without leest with pepsine diet = peanut haulm
24 h	DDM	$F_{14}^2 = 1.96$ NS	$F_{16}^4 = 7.53^{***}$ NS	$F_{23}^3 = 23.48^{***}$ $F_{11}^1 = 5.19^*$	$F_{15}^5 = 0.46$ NS	$F_{11}^1 = 1.68$ NS
	NDFD	$F_{11}^2 = 0.01$ NS	$F_{15}^1 = 0.75$ NS	$F_{11}^1 = 5.19^*$ NS	$F_{15}^1 = 0.03$ NS	$F_{11}^1 = 1.76$ NS
8 h	DDM	$F_{16}^1 = 2.18$ NS	$F_{16}^3 = 1.56$ NS	$F_{11}^1 = 1.44$ NS	$F_{12}^1 = 0.08^{**}$ NS	$F_{10}^1 = 0.56$ NS
	NDFD	$F_{15}^1 = 2.10$ NS	$F_{17}^3 = 0.26$ NS	$F_{10}^1 = 4.15$ NS	$F_{19}^1 = 0.17$ NS	$F_{10}^1 = 0.53$ NS
2 h	DDM	-	$F_{21}^4 = 7.46^{***}$ NS	$F_{16}^3 = 1.14$ NS	-	$F_{10}^1 = 0.06$ NS
	NDFD	-	$F_{20}^4 = 6.80^{***}$ NS	$F_{12}^3 = 5.51^{**}$ NS	-	$F_{10}^1 = 0.16$ NS

DDM = Dry matter degradability  
NDF = NDF degradability.

The repeatability of nylon bag method within a trial and within an animal is good.

One bag is sufficient for an incubation time within an animal.

### 3.2.1.3 - The number of trial per sample

Comparison of trials done in same conditions gives irregular results (see table 14).

With regular porc: nylon bag, the between trials differences was not significant (PC 0,05) . .

Pepsine second stage treatment removed significance ( $P \leq 0,05$ ) to between trials differences.

Finally the between trials differences have not been significant for the majority of trial groups (see table 14). However it is suitable to do two trials per sample.

### 3.2.2 - Post incubation pepsine treatment

The bag contamination by micro organisms in the rumen has been identified as a limiting factor to nylon bag method accuracy.

After rumen incubation, UDEM and VAN SOEST, 1984 treated bag with neutral detergent solution, while CHENOST in 1970 used pepsine to depress microbial contamination effect and better nylon bag technique's reproducibility.

Behrez and Orskow in 1977 used diaminopimelic acid (DAPA) to show a bag microbial contamination of minor importance.

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Table 15 : Influence of pepsine on dry matter and NDF degradability of rice straw

Incubation time (hour)	bag	Dry matter degradability p.100			NDF degradability p.100		
		Mean	Standard deviation	Number of trials	Mean	Standard deviation	Number of trials
24	Treated with pepsine	41.0	5.1	12	29.7	6.5	12
	Non treated with pepsine	39.6	3.7	12	29.9	3.3	12
43	Treated with pepsine	57.3	4.3	11	51.2	5.9	11
	Non treated with pepsine	57.4	2.61	10	53.1	6.4	11
72	Treated with pepsine	65.2	...	11	61.5	7.3	10
	Non treated with pepsine	61.4	7	11	56.9	6.6	12

Table 16 : The pepsine effect on dry matter and NDF degradability of rice straw  
Analysis of variance

Incubation time	F	Dry matter degradability		NDF degradability	
24 h		$F_{23}^1 = 0.61$	NS	$F_{23}^1 = 0.00$	NS
48 h		$F_{20}^1 = 0.00$	NS	$F_{21}^1 = 0.52$	NS
72 h		$F_{27}^1 = 1.99$	NS	$F_{21}^1 = 0.71$	NS

**Table 17 :** Dry matter and cell wall (NE<sup>1</sup>) degradability of rice straw variations with different experimental conditions (see legend)

Experimental condition		1	2	3	4	5	6
Incubation time	DDM p.100	Incubation time					
		1	2	3	4	5	6
0	DDM p.100	24.05 ± 0.92 n = 5	24.27 ± 2.59 n = 8	23.30 ± 3.12 n = 8	23.30 ± 3.12 n = 8	23.30 ± 3.12 n = 8	22.88 ± 1.28 n = 4
	NDFD p.100		12.57 ± 3.14 n = 4	13.48 ± 3.92 n = 5	13.43 ± 3.92 n = 5	13.48 ± 3.92 n = 5	13.48 ± 3.92 n = 5
24 h	DDM p.100	29.46 ± 2.90 n = 15	39.7 ± 6.10 n = 21	37.04 ± 4.81 n = 18	34.92 ± 3.40 n = 7	41.05 ± 4.94 n = 12	30.30 ± 1.6 n = 10
	NDFD p.100	25.90 ± 3.6 n = 11	31.04 ± 5.76 n = 18	11.34 ± 3.98 n = 14	23.1 ± 7.26 n = 6	29.79 ± 6.30 n = 12	
48 h	DDM p.100	45.50 ± 8.70 n = 7	58.15 ± 4.53 n = 20	56.60 ± 2.75 n = 13	53.59 ± 4.37 n = 10	57.30 ± 4.13 n = 11	34.36 ± 1.4 n = 10
	NDFD p.100	49.71 ± 10.03 n = 6	52.59 ± 6.11 n = 20	50.56 ± 6.98 n = 15	47.6 ± 5.30 n = 11	51.25 ± 5.66 n = 11	
72 h	DDM p.100	62.20 ± 5.25 n = 3	67.81 ± 5.22 n = 21	62.16 ± 6.47 n = 16	57.80 ± 5.52 n = 6	65.21 ± 6.1 n = 11	45.68 ± 4.55 n = 10
	NDFD p.100	61.93 ± 4.14 n = 3	66.11 ± 5.54 n = 17	53.99 ± 5.36 n = 12	55.45 ± 6.05 n = 11	51.51 ± 6.99 n = 10	

## LEGENDE

Trial group number	Figure	Experimental conditions
1	3	Pore size of bag = 30 microns Size of bag = 10 x 20 cm With leat Diet = peanut haulm
2		Pore size = 10 - 80 microns Size of bag = 10 x 20 cm With leat Diet = peanut haulm
3	Δ	Pore size = 10 - 80 microns Size of bag = 10 x 15 cm Without leat Diet = peanut haulm
4	j	Pore size = 10 - 80 microns Bag size = 10 x 15 cm With leat Diet = peanut haulm
5	%	Pore size = 10 - 80 microns Bag size = 10 x 15 cm Without leat Pepsine treatment Diet = peanut haulm
6	x	Pore size = 10 - 80 microns Bag size = 10 x 15 cm With leat Composed diet

We didn't find any significant difference between pepsin treated and non treated bag (see table 15 and 16).

A good washing by massage of bag under running water untill the outflow water is clear, allows suppression of post-incubation treatment.

### CONCLUSION

For characterization of feedstuff's nutritive value, based on ruminal nutrient availability, and screening of production diets, the nylon bag technique is very use ful.

However, some variation factors limit it ' s accuracy

- the pore size of nylon material, and the lest have a significant influence on nutrients degradability at the beginning of incubation. Their influence on final extent of degradation is inexisting.

Nylon material with regular pore size, gives less variable results; the lest limits participation of bag to gastric movements and may influence negatively the "in situ" degradation of nutrients at the firsts moments of incubation.

With forage based diet, bags are closely associated with digesta wich prevents their floating in the rumen.

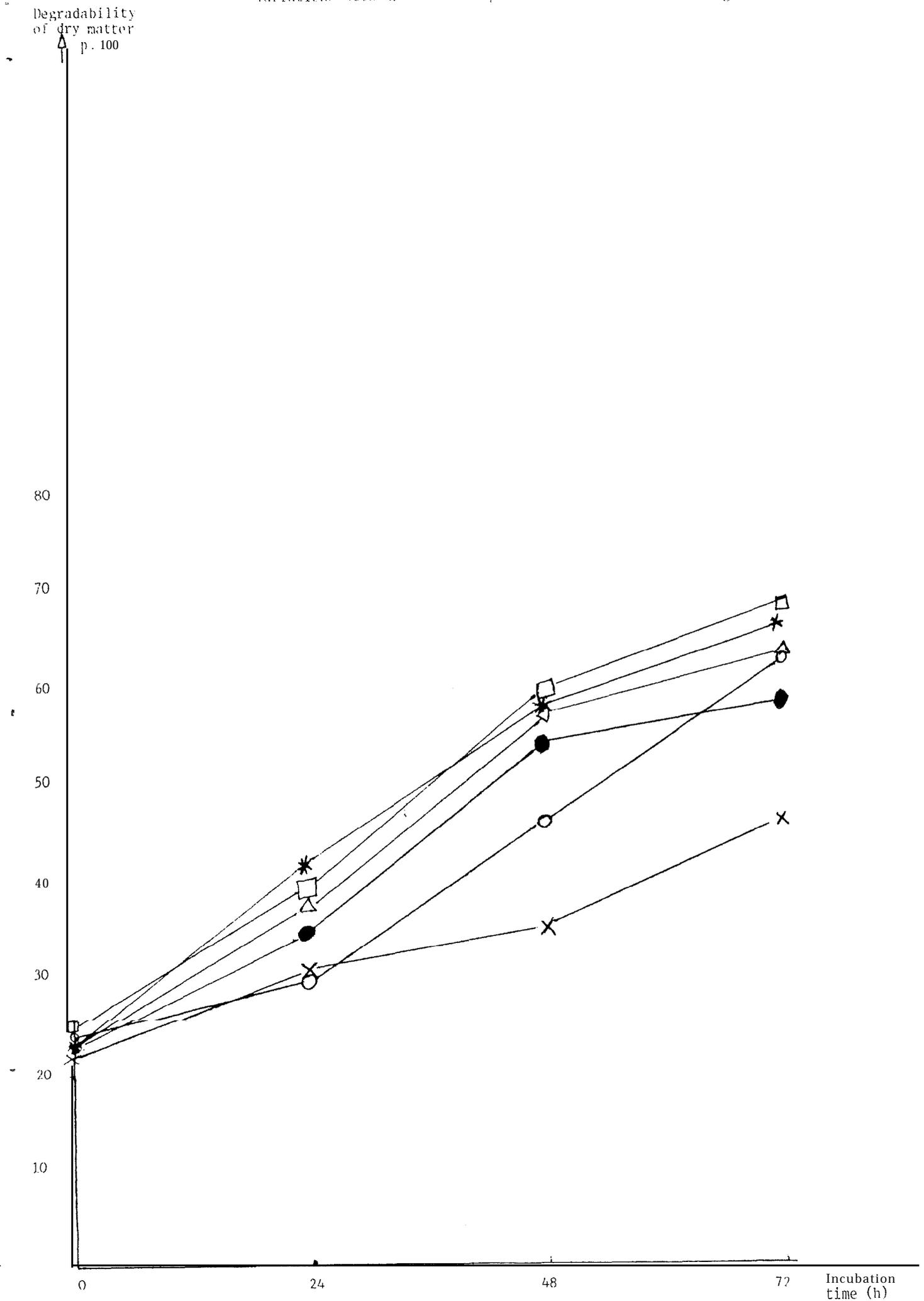
The bag attachment on a plastic tube according to Orskov's model is quite working.

- A good choice of animal diet is of major importance for nutritive value evaluation of feedstuffs. A good quality forage with constant nutritive value is required. Energetic and protein supplement based on oil cakes is often necessary for animal growth and to create optimal ruminal conditions for cellulose and protein degradation. This can be checked by determination of rumen fluid pH, level of ammonia and volatil fatty acids.

- The between animals and trials differences are significant while the between bags differences are not.  
A sufficient number of repetitions is required to have reliable mean values per sample.  
It is advisable to work with not less than three animals and to do two trials per sample. For each incubation time a duplicate bag is not necessary.
- Results between sheep and cattle are not significantly different.
- Bag size has no influence on nutrient degradability.  
However it must be large enough to allow free movement of sample and digesta at the same time.
- Our test sample, rice straw is poor in nitrogen. Minerals, essentially made of silica, are not available.  
It's high level of cellulose is an available energetic source (see table 17 and figure 4) in spite of some variations

"In situ" dry matter degradability of rice straw at 48 hours of incubation is close to in vivo values. It confirms CHENOST's observations who studied forages degradability in 1970.

Figure 4 : Dry matter degradability of rice straw  
Variations with different experimental conditions (see legend)



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