Use of *in vitro* gas production technique to assess the contribution of both soluble and insoluble fractions on the nutritive value of forages

by

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A mi familia.

DECLARATION

This is to declare that this thesis has been composed by me and has not been presented in any previous application for a degree. All sources of information are shown in the text and listed in reference and all help by others have been duly acknowledged.

R. M. Pedraza

ABREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
AOAC	Association of Official Agricultural Chemist
C	Celsius
CP	Crude protein
degrad	degradability
deterg	detergent
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
h	hour
FIR	Filter paper insoluble residue
IFRU	International Feed Resources Unit
ME	Metabolizable energy
min	minute
NDF	Neutral detergent fibre
NDIR	Neutral detergent insoluble residue
NFE	Nitrogen free extract
PEG	Polyethylene glycol
PF	Partitioning factor
PVP	Polyvinylpolypyrrolidone
SCFA	Short chain fatty acids
SIR	*in sacco* insoluble residue
VFA	Volatile fatty acids
N	Nitrogen
SD	Standard deviation
WLR	Washing loss residue

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SUMMARY

The objective of this work was to characterise the *in vitro* gas production contribution of both soluble and insoluble fraction in forages. Four forages were used: 1) ***Gliricidia sepium*** leaves, 2) sugar cane tops, 3) Barley straw and 4) hay (mixture of legumes and grasses). Three separation methods were applied: 1) Washing of sample in a nylon bag using washing machine, 2) washing the sample trough a Whatman No. 1 filter paper and 3) washing the sample using detergent solution, as described by Goering and van Soest (1970). The equation $p= a+ b (1 – e^{-ct})$ was utilised to fit rumen degradability and *in vitro* gas production data. The initial characterisation of the forages showed significative differences ($P < 0.001$) in 48h rumen degradability and rate of degradation, as well as *in vitro* gas production at 48h and rate of gas production. All washed samples showed losses of soluble material (ash included) and also small particles from the nylon bag washing method. In general Gliricidia had the greatest washing loss and straw the least. The gas production was in general significantly higher for the unwashed forages. Gliricidia gave the highest values of gas production and straw the lowest. The rate of gas production in the insoluble fraction between methods was similar in *in sacco* and filter paper, while detergent solution tended to be lower. The gas production of the insoluble Gliricidia fraction was lower than the other forages, while it reached the highest values from the fermentation of its soluble fraction. Such data suggests that the contribution of the fibrous material on the nutritive value of Gliridicia may be limited. Therefore, the role of Gliricidia in supplying readily fermentable fibre and improve rumen function should be studied in more detail. The results indicated differences between separation methods. It was noted some negative influence of high ash concentration in the soluble fraction on *in vitro* gas production. This influence seems to be greatest on forages with small washing losses. The results suggested that it is generally more appropriate to measure the degradation of organic matter as usual dry matter can give problems of interpretation of the significance of the soluble fraction.

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1.0- Introduction

Nowadays our world still full of contradictions. While man can build technologies to search other planets and even to destroy ours, he is not able to provide enough food for all. The increase in population, the environmental degradation, the every day increasing differences between rich and poor, increasing illiteracy, the unsustainable way of life of some societies, local wars, unfair trade agreements, the use of food as an instrument of political coercion and the death of millions from undernutrition and associated diseases every year are some of the legacies which mankind will leave for the next century.

In this scenario, and knowing that the solutions to those problems will have to deal unfortunately with several political and economical interests, the agriculture and in particular the agricultural sciences should accept the challenge for the use, research, development and teaching of technologies that involves the maximum use of local resources with an ecological and economical sustainable approach. Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs (WCED, 1987).

Animal agriculture is one of the most important components of global agriculture and livestock is one of the main users of the natural resources base (de Haan *et al*, 1996). Ørskov and Viglizzo (1994) have stressed also the role of the animals in spreading farmer's risk, which in some countries is a matter of family survival.

The ability of ruminants to use fibrous material, a widely available resource, matched with the sharp increase in the population, mainly in developing countries, and therefore increasing the demand for food. In opposition to that in many developed countries, foods that are suitable to for human comsuption are very often used for both monogastric and ruminant animal feeding. The ruminants should be fed, as far as possible, on roughages and others feeds that are not in competition with human food (Ørskov, 1998).

The rumen contains a very well adapted microbial population in order to utilise cellulosic materials that will be later used by the host animal. Qualitative observations on the presence of rumen bacteria and protozoa, and volatile fatty acid production as a result of the foodstuff fermentation in the rumen, were first made in the 19th century. It was not until the 1940s that workers in Cambridge, Phillipson (1942), Barcroft *et al*., (1943) and Barcroft *et al*., (1944), did quantitative experiments showing the nature of the acids formed in the rumen as well as their absorption and relations to the energy needs of ruminants.

Most forages and fibrous by-products can be solely used as food products for humans if they first "pass" through the animals, mainly ruminants. The animals utilise part of this food and offer valuable products to human nutrition. In the search to improve and optimise this "trophic chain", it is therefore crucial to further develop methods to evaluate fibrous feeds, so that ruminant production can be maximised.

1.1 - Objectives.

The main objective of this work is to characterise the *in vitro* gas production contribution of both soluble and insoluble fraction of forages and to compare different methods to separate both fractions.

2.0- Literature review.

2.1 - Estimation of nutritive value of ruminant feeds.

Evaluation of feeds should provide nutritionists with the necessary information to formulate a diet from both a physiological and an economical point of view, in order to optimise the animal performance. The need for precise nutritive characteristics of ruminant feeds should be ever greater in the future owing to: a) advances in molecular biology, particularly the development of transgenic plants; b) crop improvement programmes; c) environmental and economic constraints concerning the use and/or disposal of crop by-products and residues (Theodorou *et al*., 1994).

Laboratory methods to estimate nutritive value of feed have improved since the first ideas in 1725, when ruminant feeds were evaluated as Straw Units (Blaxter, 1986). Initially, the techniques were designed mainly to characterise nutritive value of the foodstuff rather than to predict animal performance. Improvements in the methods of food evaluation have followed new concepts in chemistry, animal physiology, rumen microbiology knowledge and related fields of science (Flatt, 1988).

Feed evaluation needs to define roughage characteristics which determine animal performance, for example liveweight gain, milk yield, wool growth, etc (Blümmel *et al*., 1997a). Of particular relevance is the prediction of the animals intake, which is an important aspect related to feeding forages (Minson, 1990). In practice, the prediction of the roughage intake still presents problems (Blümmel and Becker, 1997).

The future development of feed evaluation systems should incorporate new information on the relationship between specific end-products of digestion and performance of the animals, as well as information on animal and microbial metabolism, feed composition, and effects of various factors on feed utilisation (Flatt, 1988). An adequate dietary analysis of any sort requires that the methods employed are relevant to a nutritional classification of the dietary chemical components (Van Soest and Robertson, 1985). The current achievements in this field show a very

exciting new horizon in feed evaluations, with particular emphasis on roughages evaluation (Ørskov, 1993a).

In the evaluation of ruminant foodstuffs, different methods are currently available. Some of these methods will be considered in this review.

Nowadays, the *in sacco* technique is probably the most widely used method for feed studies, however some drawbacks have been pointed out (Michalet-Doreau and Ould-Bah, 1992). Others procedures are also used; proximate analysis, its alternative procedure for the fibre (Van Soest, 1967; Goering and Van Soest, 1970) and modern instrumental techniques. The solubility test, the two-stage technique (Tilley and Terry, 1963), the enzymatic method (Jones and Hayward, 1975), the rumen simulation technique "RUSITEC" (Czerkawski and Brekenridge, 1977), as well as the gas production technique (Menke *et al*., 1979; Menke and Steingass, 1988).

2.2- The *in sacco* degradability.

In sacco method, also named as nylon bag or *in situ*, is based on depositing separately foodstuff into bags which are incubated into the rumen of an animal fitted with a rumen cannula. The main objective is to measure the disappearance of dry matter and/or other nutrients. In early experiments (Quin *et al*., 1939) silk bags were used to incubate samples. These were latter replaced by other types of clothes, e.g. nylon, polyester or Dacron.

Initially the method was successfully used to assess different foodstuffs and to determine the effects of formaldehyde treatment on degradation of protein supplements (Erwin and Elision, 1959; Rodríguez, 1968; Demarquilly and Chenost 1969; and others). Results from such studies were obtained from a single incubation time, e.g. 24 and 48 hours, which as later were shown to have some drawbacks.

In 1977, Mehrez and Ørskov proposed the use of the *in sacco* methods as a routine procedure for measuring protein degradation rate, incubating several bags in order to obtain the kinetic evaluation of the degradation. Van Soest *et al*., (1978) and Ørskov

et al., (1988) have suggested the use of kinetics of fermentation data to improve the estimation of nutritive value of feeds when both *in vitro* and *in sacco* methods are considered. Such a dynamic approach improves markedly the potential of this technique, as suggested by Ørskov *et al*., (1980) in forage evaluation.

2.2.1. Sample size to bag surface ratio.

The ideal ratio has been quoted as about 15 mg DM/cm^2 (Ørskov, 1992 and Michalet-Doreau and Ould-Bah, 1992). The incubated sample should be able to move freely within the bags to avoid formation of microenvironments that affect replication of the analysis. Other aspects that have to be considered in sample bag size ratio is the amount of residue required for further analysis. The number of bags at one given time should depends on the species of the host animals.

2.2.2- Bag pore size.

The ideal pore size shall allow the entrance of rumen liquor and microorganisms, i.e., protozoa, bacteria and fungi, and the efflux of degraded fraction and microorganisms. Bag pores should also be sufficiently small to minimise losses of undegraded feed particles, while maintaining an active microbial population and to preventing blockage of the pores by feed components. The choice of bag porosity must be a compromise between loss of undegraded feed particles and the movement of microorganisms through the bag. A pore size between 40-60μm is adopted as a standard (Ørskov, 1992 and Michalet-Doreau and Ould-Bah, 1992).

2.2.3- Diet of the host animal.

The ideal condition in evaluating one feed is that the diet should be similar to that which is under evaluation (Ørskov, 1992). However when it is necessary to evaluate several samples in one experiment this is a limiting factor. Therefore it should be a formulated diet that ensures an optimal rumen environment for microbial growth, with particular emphasis in cellulolytic bacteria. A stable rumen environment is the most important constrain in that case.

In the evaluation of protein supplements of vegetable origin it is noted that when diet changed from a high concentrate to a high roughage there was a significant decrease in degradation rate, nevertheless only small differences were recorded when protein supplements of animal origin were analysed (Ørskov, 1992).

2.2.4- Number and species of animals.

Mehrez and Ørskov (1977) observed that the greatest source of variation of the *in sacco* technique was the host animals. It has been suggested that a sample has to be incubated for at least two periods in three animals to give an accurate estimation for one given incubation time. There are small or no differences in the *in sacco* degradation rate from samples incubated in either sheep or cattle. The use of cattle has the advantage of allowing a large number of bags or larger size of bags per period (Ørskov, 1992).

2.2.5- Sample preparation.

As far as possible the sample should represent the materials, as they would appear in the rumen had they been consumed by the animals. Therefore, the ideal sample preparation would be a masticated digesta from animals fitted with oesophageal cannula (Ørskov, 1992). As this is not feasible in most cases it is suggested that dry and milled sample is used instead. The dry sample should be milled through a 2.5-3.0 mm diameter sieve. The green forages and silage should be processed using a mincer with 5.0 mm screen (Ørskov, 1992). Mehrez and Ørskov (1977) and Nocek (1985) recommended to pre-soak bags containing the sample in water, although limited information is available on the advantage of such procedure (Michalet-Doreau and Ould-Bah, 1992).

2.2.6- Incubation and withdrawal of the bags.

Bags should move freely within the digesta, both in the liquid and solid phase (Ørskov. 1992). The inclusion of an extra weight to ensure proper anchorage does not seem to be of major importance (Michalet-Doreau and Ould-Bah, 1992). The time that each bag stays in the rumen will depend on the characteristics of the sample. It is important that the most sensitive part of the curve is well supported by observations (Ørskov, 1992).

2.2.7- Washing the bags.

In order to eliminate other food particles and microorganisms the bags can be washed by hand or machine (Michalet-Doreau and Ould-Bah, 1992). The residue is dried to determine the losses during the incubation time. Food samples should be placed into more than two different bags, then washed in the same way in order to determine the "washing loss". Such analysis should provide information on the presence of very fine particles and/or very rapidly degradable fraction in the sample.

2.2.8- Interpretation of results.

It is assumed that sample disappearance is synonymous of degradation. Although this is generally true, there are several cases where the assumption is not valid. For example, substrates with fast disappearance, i.e. water-soluble materials (Ørskov, 1992). The degradation curve should show the sample degradation with time. The curve may be expressed mathematically using the equation proposed by Ørskov and McDonald (1979):

$$p = a + b\,(1 - e^{-ct})$$

Where p is the percent of degradation at time t, a is the soluble fraction, b is the insoluble but potentially degradable fraction. The a + b value is the potential of degradability of the material, all expressed in percentage, and c is the degradation rate, expressed in percentage/h.

The extent of protein breakdown will depend upon the time for which the protein remains in the rumen. Ørskov and McDonald (1979) pointed that the effective degradability P may then be defined as:

$$P = a + [bc/(c+r)] [1 - e^{-(lc+r)t}]$$

In which r is the rate of passage from the rumen to the abomasum. As the time of incubation increases, the fraction of the protein remaining in the rumen falls to zero, as does the rate of breakdown, and P may then be defined as follows:

$$P = a + bc/(c + r)$$

In this equation a is the immediately degraded protein and bc/(c + r) the slowly degradable fraction. The value of r may be determined by treatment of the protein with dicromate.

The a, b and c values are used to determine the feed potential of forages as well as to recommend feeding strategies. Feed potential indicated the consumption of digestible energy relative to maintenance (Ørskov, 1993a; IFRU, 1998). An index value is equated with the feed potential and indicates the relative consumption or performance possible from the foodstuffs. More information is required to substantiate such an approach, but so far it seems promising with information from different places with large differences in feed resources and types of animals (Ørskov, 1998).

Tests carried out at several laboratories have shown unacceptably high between laboratory variability, indicating a need for more strict procedures which have to be rigidly adhered to if the results are to be universally applied in practice (McDonald *et al*., 1995).

2.3- Chemical analysis.

Chemical analysis is the simplest way to evaluate foodstuff. Much of the existing information about the composition of food is based on the proximate analysis of foods, which was developed over 100 year ago by two German scientists, Henneberg and Stohmann (McDonald *et al*., 1995). Frequently the proximate analysis is also called the Weende procedure, in honour of the Weende Experiment Station, were the experiments were carry out. Today it has been partially replaced by other analytical procedures.

The proximate analysis divides the food into six fractions: moisture, ash, crude protein, ether extract, crude fibre and nitrogen free extractives. Moisture content is determined by drying the sample to constant weight at 100°C, and ash contents by burning of the sample at 550° C until all carbon has been eliminated. Crude protein is calculated from the nitrogen content, assuming that the all the nitrogen of the food is present as protein and that all food protein contains 160g N/kg (McDonald *et al*., 1995), then CP = total N x 6.25. This assumption is not always true, but it is assumed the 6.25 factor as correct because the in many domestic animals the protein requirements are also expressed as N x 6.25.

The ether extract fraction is determined by subjecting the food to a continuos extraction with petroleum ether for a fixed period, as well as lipids such a fraction contains organic acids, alcohols and pigments. The crude fibre value is obtained by subjecting the sample to successive treatments with boiling acid and alkali solutions; the organic residue is the crude fibre. The nitrogen free extract is calculated by subtraction from 100 of the sum of moisture, ash, crude protein, ether extract and crude fibre, expressed in percentage. In recent years the proximate analysis procedure has been severely criticised as being archaic and imprecise, most criticism have been focused on the crude fibre, ash and nitrogen free extractive fraction (van Soest, 1982; McDonald *et al*., 1995).

The alternative procedure for fibre proposed by van Soest (1967) is well accepted today. It was developed as a procedure to quantify both the cell contents and the cell wall constituents mainly in vegetal materials. The neutral detergent fibre (NDF) is the

residue after extraction with boiling neutral detergent solution and it consists mainly of lignin, cellulose and hemicellulose and can be taken as a measure of the plant cell wall. The acid detergent fibre (ADF) is the residue after refluxing with sulphuric acid and cetyltrimethyl-ammonium bromide and indicates the concentration of lignin, cellulose and silica.

The term ash is imprecise in itself and it takes into account both minerals and silica fractions. The determination of ash is the stating point to the measurement of individual minerals. Different instrumental methods support nowadays these analysis, from atomic absorption spectrophotometer to the induced couple plasma apparatus.

The NFE, also referred to as "soluble carbohydrates", includes both soluble and insoluble carbohydrates, as well as all the errors in the determination of the other fractions. The van Soest method is far more accurate in determining the cell wall constituents.

2.4 – *In vitro* methods.

The use of *in vitro* techniques is rapidly expanding because of an increased need for routine and reproducible methods to obtain data on bioavailability of feeds in addition to chemical ones (Gizzi *et al.*, 1998). Since digestibility trials are laborious and expensive to perform, there have been numerous attempts to determine the digestibility of foods by reproducing in the laboratory the reactions which take place in the alimentary tract of the animal (McDonald *et al.*, 1995).

The two stage technique (Tilley and Terry, 1963) is employed in many forage evaluation laboratories and involves two steps in which the forages are subjected first to a fermentation *in vitro* with rumen fluid followed by a digestion with pepsin in weak acid. Monson *et al.*, (1969) evaluated the *in sacco* and the two stage *in vitro* digestion and it was concluded that when these methods are properly used can be a valuable assistance in evaluating the quality of large numbers of forage samples.

The two-stage technique (Tilley and Terry, 1963) was developed as an end-point digestibility method and thus, unless lengthy and labour intensive time-course studies are completed, the technique does not provide information on the kinetics of forage digestion (Theodorou *et al*., 1994). Omed *et al*., (1998) pointed out that the need for carbon dioxide; a centrifuge and pepsin are important drawbacks for the application of such method in the developing countries.

As with the Tilley and Terry method, enzymatic methods of evaluation are routinely used as end-point digestibility procedures, but also do not provide information on kinetics of forage digestion. The main advantage of enzymatic methods is that it does no require animals as inoculum donors (Theodorou *et al*., 1994). The use of the neutral detergent fibre instead of the enzymatic treatment can reduce the time required for the analysis (Menke *et al*., 1979).

Van Soest and Robertson (1985) summarised as follow the lack of ruggedness of three of these methods related with the reagent use:

Method	**Problems**	**Reagent characteristic**
Tilley and Terry	Many steps	Rumen fluid, a variable reagent
Protein solubility	Confoundation with NPN	Unstable buffer reagent
Protein degradability	Enzyme reagent is limiting	Protease

The rumen simulation technique (Rusitec) was developed at the Hannah Research Institute (Czerkawski and Breckenridge, 1977). In the Rusitec, solid digesta is contained in monofilament nylon mesh bags inside a perforated cage, which slides inside a cylindrical reaction vessel. Artificial saliva and rumen inoculum are infused into the vessel and the excess liquid and the fermentation gases, forced through an overflow by small positive pressure, are collected separately. Thus, the free microbial solution is separated physically from microorganism associated with the mass of solid digesta, but a continued interaction between these two microbial systems is facilitated

by the coarseness of the nylon bags and the perfusion of the solid by the liquid. As described by Cheng and McAllister (1997) Rusitec constitutes a rumen without wall. The absence of absorption across a semipermeable membrane (rumen wall) is probably responsible for most of the inconsistencies that exist between measurements of fermentation characteristics made in Rusitec and those made *in vivo* (Mansfield *et al.*, 1995). Nevertheless, the Rusitec continues as an invaluable research tool for the study of the rumen metabolism and ruminant foodstuff.

2. 4- *In vitro* gas production technique.

The association between rumen fermentation and gas production has long been known, in an early attempt in 1939 was tried to record the gas production directly via the rumen cannula in sheep, the technique was too difficult to execute and of poor reproducibility (Blümmel *et al*., 1997). In 1974 Menke and Ehrensvärd (cited by Theodorou *et al*., 1994) studying the stoichiometry of rumen fermentation with a syringe closed system noticed a very reproducible gas production data when the same substrate was incubated in the same quantity in different runs. In the system published by Menke *et al* (1979) the substrate is incubated in a calibrated gas-tight glass syringe fitted with a plunger, the gas produced over 96 h period is recorded. The incubation media include rumen liquor and a buffer. The main innovation in such method was that the gas production is recorded rather than degradation. The same postulate continues until today, even when the method had been simplify and improved.

2.4.1- Sample preparation and size.

The pioneer paper (Menke *et al*., 1979) referred to the fact that there is a high correlation between the amount of substrate and the gas produced over time. As a guide it is suggested that no more than 200mg of dry matter of sample should be used, but if it is poorly digestible, it could be 300g (Menke and Steingass, 1988). In a 24h gas production procedure applied before determine microbial biomass yield (Blümmel and Becker, 1997) it utilised 500 mg of sample. Substrates should be milled using a 1mm screen to allow more precise sampling. Gerson *et al*., (1988) reported the effect of particle size, i.e. 0.1-0.2 mm to 1.0-2.0 mm, on *in vitro* gas production.

The *in vitro* assay of foodstuffs in rumen fluid requires a careful drying (Menke and Steingass, 1988). Rymer and Givens (1998) observed that high temperature dried grass negatively affects the gas production profile. Nagadi *et al*., (1998) observed that the gas production profile of ***Festuca rubra,*** dried for 20 h at 65° C, is more closely associated with that of the fresh sample when milling is completed through 0.4 mm sieve as compared to 1.0 mm.

Deaville and Givens (1998) suggested that it is preferable to use pre-dried silages to avoid interference from any indirect gas produced. The result indicates that fermentation acids in silage do not yield direct gas and therefore provide little energy for rumen microbial growth, and the contribution of fermentation acids to diet/feed ME content should be subtracted during the calculation of fermented metabolizable energy.

2.4.2- Buffer and inoculum.

The ideal ration buffer-rumen fluid should be 2:1 when comparing samples (Menke and Steingass, 1988), but it should not be if its necessary to study the inoculm *per se.* The buffer should be able to neutralise the volatile fatty acids produced during fermentation in order to keep a constant pH. Secondly, the buffer should supply all necessary minerals for optimal microbial activity. The quantity of gas produced during *in vitro* fermentation is a result of from gaseous end-products, i. e. CO_2, CH_4 and H_2, from substrate fermentation as well as CO_2 released from the buffer. Such CO_2 is a result of rumen microorganisms lysis and volatty fatty acids production (Menke *et al*., 1979). It is recommended to take the rumen fluid before feeding, because it is most constant in its composition and activity. It is also advisable to take the rumen fluid mixture from at least from two donor animals as this guarantees a greater constancy of activity (Menke *et al*., 1979; Menke and Steingass, 1988).

2.4.3- Incubation conditions and time of reading.

Incubation vessels, *i. e.* syringes, bottles, should be kept in a water bath at 39 ±0.5° C. The time can be selected to suit the type of substrate which is being incubated. For forages, it is generally accepted to read after 3, 6, 12, 24, 48, 72 and 96h but for concentrate type substrates it may be necessary to take more frequent readings in the first 24h (Menke and Steingass, 1988). Like the *in sacco* technique, it is important that the most sensitive part of the curve is well supported by observations

2.4.4- Applications and improvements of the method.

The quantity of gas produced from the *in vitro* incubation of a substrate is closely related to its digestibility and consequently to its energetic value (Menke *et al*., 1979; Menke and Steingass, 1988). The gas production data may provide a prediction of the effective organic matter degradation in sacco (Deaville and Givens, 1998a). The first application of the *in vitro* gas technique was to evaluate the energetic value of foodstuffs. Nevertheless, this technique has also been used to assess the presence of antinutritives compounds in different foodstuffs, *e. g.* browse plants.

Various papers have been published recently on the use of the gas production technique as a method to study antinutritive factors. The use of tannin binding complex agents, *e. g.* insoluble polyvinylpolypyrrolidone, was first proposed by Andersen and Sowers (1968) as an indirect method to determine biologically active tannin in plant materials. Makkar *et al*., (1995) did the first work on the use of tannins binding complex agents in the *in vitro* gas production to evaluate antinutritive factors. Further works is this aspect had been published by Khazaal and Ørskov (1994), Khazaal *et al*., (1994), Wood and Plumb (1995) and Khazaal *et al*., (1996). The main drawbacks of such an approach are that the response to tannin complexing agents procedure seems to be more effective in food with high tannins concentration and the effects of other antinutritives factors, apart from tannins, can’t be study.

Several studies have been completed to improve and/or broaden the use of this technique as a tool in the field of the animal nutrition. Such research has mainly

focused on improving the buffer, standardising the inoculum, processing the sample and automating the technique. Despite the relevant results achieved further research in these areas is required.

Altaf *et al*., (1998) and Mauricio *et al*., (1998a) showed that faecal material has a potential use as an alternative inoculum to rumen liquor for the gas production technique. This will avoid the use of cannula and/or catheters to facilitate the inoculum collection. Rymer *et al*., (1998b) observed that some Nepalese grasses were fermented more rapidly and completely when rumen fluid from buffalo was used, as compared to sheep rumen fluid. This paper also referred the necessity of standardisation and the influence of the inoculum source. Van Larr *et al*., (1998) found differences in fermentation characteristics between hull and endosperm of full fat Soya beans, this is possible related to the polysaccharide composition, mainly the differences in cellulose, hemicellulose and pectin concentration in each fraction. This work pointed out the necessity to assess the different vegetal fractions in a sample.

The use of an automated procedure for measuring gas production *in vitro* has been reported (Theodorou *et al*., 1994; Cone *et al*., 1994; Mauricio *et al*., 1998b and Fakhri *et al*., 1998). The use of a pressure transducer in combination to a computer resulted in a reduced labour requirement. This seems to be an appropriate approach when large numbers of samples are being analysed on a routine basis.

2.4.5- Prediction of *in sacco* and *in vivo*.

Menke and Steingass (1988) observed a close correlation between *in vitro* gas production and digestibility; the better correlation was achieved when the equation includes crude protein, crude fat and ash content.

Blümmel and Ørskov (1993) adapted gas production technique to describe the kinetics of fermentation based on the exponential model $p = a + b (one - e^{-ct})$ and to predict feed intake in cattle. The results showed that total gas production (a + b) value as described by the equation were correlated with intake (0.88), digestible dry matter intake (0.93) and growth rate (0.95) in a multiple regression model. The use of the rate of gas production c value did not improve the precision of the correlation. Tuah *et*

al., (1996) evaluating several local Ghanaian foodstuffs and found a positive and significant correlation (0.58 to 0.95) between *in sacco* DM degradability and *in vitro* gas production**.**

Blümmel and Becker (1997) explored the potential of an *in vitro* gas production for the estimation of rates and extent of fermentation of roughages and roughage neutral detergent fibres and the relevance of these estimates for the prediction of dry matter intake. The results showed that an average gas production from NDF measured from 24-96 h accounted the 81% of dry matter intake and only the 75% of intake were accounted by the whole forage gas production. Blümmel *et al*., (1997b) evaluating the relationship between gas production, *in vitro* apparent true DM degradability in forty-two roughages found that roughages producing proportionally less gas per unit substrate truly degraded had higher feed intakes.

Blümmel and Becker (1997) used various parameters of the *in vitro* gas production to predict the DMI of 54 forages. In the gas test were used both the whole sample and the isolated NDF fraction and two exponential models. Approximately 69% of the variation of DMI were accounted for by the combination of the potential cumulative gas production and the rate of gas production. Nevertheless, the kinetics parameters based on NDF incubation explained about the 81% of DMI. Combinations of *in vitro* true degradability at 36 or 48 h with gas volumes measure in the same incubation either at 4, 6 or 8 h accounted for approximately 82 % of the variation of DMI and the inclusion of the PF improved the correlation to 87%.

Khazaal *et al*., (1993) correlated chemical composition (*i.e*. CP, NDF, ADF or ADL) with *in vitro* two-stage digestibility, *in sacco* degradability and gas production with voluntary intake and *in vivo* apparent DM digestibility of 10 graminaceous hays in sheep. Accurate prediction of intake and *in vivo* apparent DMD were achieved using NDF, ADF, ADL, and CP in multiple regression. However, using the a + b and c of gas production only intake was predicted accurately. The lower performance of the gas test was attributed to the small contribution to gas production and higher buffering capacity resulting from protein fermentation.

The partitioning factor (PF) is a concept first proposed by Blümmel *et al*., (1994) which represents the ratio of substrate truly degradable to volume of gas produced at a given incubation period, usually 24 or 48 hours. Such index reflects the variation in biomass yield and it is expressed in g/L. The authors observed that PF was highly correlated with DMI.

Blümmel and Bullerdieck (1997) calculated the partitioning factor at 24 and 48h incubation for published data on *in vitro* gas production, *in sacco* degradability and DMI. Again, a good correlation was observed between partitioning factor and DMI. However, the use of both rate and extent of *in sacco* or *in vitro* gas production as extra variables did not improve the correlation. This was probably due to an interrelation between rates of fermentation and PF. In general, there is a positive correlation between gas production and VFA production, and a negative correlation between gas production and microbial biomass yield (Blümmel *et al*., 1994).

Blümmel *et al*., (1997a) completed the negative correlation between gas production and SCFA with microbial biomass yield. It was concluded that the partitioning between SCFA and microbial cell production is not constant. Use of *in vitro* gas production test to estimate nutritive value might select against maximum microbial yield by favouring substrates with proportionally high SCFA yield and therefore high gas production. This intrinsic disadvantage of the *in vitro* gas production technique may be overcome by combining gas measurements with determination of the undegraded residue.

3.0 - Materials and Methods.

3.1- Samples description.

Four forages were used in this study: 1) Gliricidia (***Gliricidia sepium***) leaves collected from Cape Coast, Ghana during July 1997, 2) sugar cane tops (***Saccharum officinarum***) collected from Camagüey, Cuba, in January 1998, 3) Barley straw (***Hordeum distichum***) and 4) hay (an undetermined mixture of Scottish grasses and legumes), both collected from the Rowett Research Institute; Scotland, in March 1997. Gliricidia and sugar cane tops were chopped before oven drying at 55° C for 48h.

Samples were milled in a hammer mill (Christy & Norris, size 8inch. Shelmsford, England. UK) using an either a 2.5mm or a 1.0mm screen. Samples milled through 2.5mm screen were used for *in sacco* determinations. All other analyses were carried out with samples milled through 1 mm.

3.2 - *In sacco* rumen degradability analysis.

The dry matter degradability was carried out according the procedure described by Mehrez and Ørskov (1977). Nylon bags of 160 x 85mm size with 40 µm pore size were used. Approximately 3.0g sample were transferred to the bags and incubated for 8, 16, 24, 48, 72 and 96h into the rumen of three adult sheep with a permanent cannula (Ø 60mm). The animals were fed twice a day with 67% hay and 33% grass cubes diet; the water was offer *ad libitum*. At the end of the incubation time the samples were withdrawn from the rumen and washed in a domestic washing machine with cold tap water for about 17min without spinning. The bags were then oven dried at 65°C for 48h and weighed. The washing losses were measured by washing the nylon bags containing samples with washing machine and dried as described before. All nylon bag determinations were in duplicate.

The *in sacco* degradability data were fitted into the equation $P = a + b\ (1 - e^{-ct})$ (Ørskov and McDonald, 1979), with the NEWAY software, version 5.0 (Dr. X. B. Chen. IFRU, Rowett Research Institute; Aberdeen, UK).

3.3 - *In vitro* gas production analysis.

In vitro gas production was completed according to the procedure described by Menke and Steingass (1988). Each sample, both original forages and insoluble residues, weighing about 200mg, were put into 100ml calibrated glass syringes (FORTUNA®, Häberle Labortechnik. Germany) together with 30ml rumen liquid-media solution on a 1:2 ratio. Syringes were incubated in a water bath at 39°C, where a transparent plastic lid with holes held the syringes upright. Two blanks and a standard hay sample of known gas production were included in each run.

Rumen fluid was collected from two fistulated sheep fed on hay-grass cubes diet (**section 3.2**) Rumen fluid was transferred into pre-warmed thermos bottles and strained through two layers of gauze. Rumen fluid was manipulated under continuous flushing of CO_2.

The media solution was composed as follows:

Macromineral Solution

5.7g Na_2HPO_4
6.2g KH_2P0_4
0.6g $MgSO_4 7H_2O$
make up to 1L with distilled water

Micromineral Solution

13.2g $CaC1_2 2H_2O$
10.0g $MnC1_2 4H_2O$
1.0g $CoC1_2 6H_2O$
0.8g $FeC1_2 6H_2O$
make up to 100ml with distilled water

Buffer Solution

35.0g $NaHCO_3$
4.0g $(NH4_4)$ HCO_3
make up to 1L with distilled water

Resazurin Solution

100mg Resazurin
make up to 100 ml with distilled water

Reducing Solution

2.0ml 1N NaOH
285.0mg Na_2S $7H_2O$
47.5ml distilled water

Preparation of Media Solution

474.00 ml distilled water
0.12 ml microminerals
237.00 ml buffer solution
237.00 ml macromineral solution
1.22ml Resazurin solution
warm to 38^0C then add reducing solution (this is prepared fresh for each run)

The initial gas volume was recorded and then after 3, 6, 12, 24, 48, 72 and 96h incubation. The gas productions of the soluble fractions were estimated by subtracting the gas production of the insoluble fractions from the whole sample. The gas production data was fitted into the exponential equation as described in **Section 3.2**.

3.4 – Procedures for removal of soluble compounds.

3.4.1 - *In sacco.*

The *in sacco* insoluble residues (SIR) were obtained when the samples were washed as described in **Section 3.2**. All forages were analysed in duplicate in three different runs. The SIR used for both chemical analysis and *in vitro* gas production were previously milled in a laboratory hammer mill (Culatti type DFH 48, Switzerland) through a 1mm screen.

3.4.2 - Filter paper.

All forages were analysed in duplicate in three different runs based on a modification of the procedure described by Department of Agriculture. Laboratory Manual, University of Aberdeen (1997/98). Samples of known DM content were soaked in 150ml distilled water at room temperature during 1h and 45min while shaken intermittently. Samples were then filter through a Whatman # 1 (ø 15cm) filter paper and washed with water until approximately 500ml of filtrate was recovered. The filter paper containing the insoluble residue was oven dried at 60°C for 48h. The filter papers were transferred to previously tared plastic bags and sealed. Weighing was carried out after 1h, to calculate the filter paper insoluble residues (FIR).

3.4.3 - Neutral detergent solution.

Triplicate samples were subjected to neutral detergent treatment as describe by Goering and van Soest (1970). The neutral detergent insoluble residues (NDIR) were dried at 60°C for 48h and kept for further analysis.

3.5 - Chemical analysis.

Dry matter and ash contents were determined according AOAC (1984). Sugars contents were analysed by gas chromatography (Hoebler *et al*., 1989); nitrogen in a Foss/Heraeus automated Dumas system and the NDF contents by the method of Goering and van Soest (1970).

3.6 - Statistical analysis.

The statistic analysis (ANOVA) was completed using MINITAB software, release 9.2 (Copyright 1993, Minitab. Inc.), and the differences between means by Least Significant Differences Method (Snedecor and Cochran, 1967).

4.0- Results and Discussion.

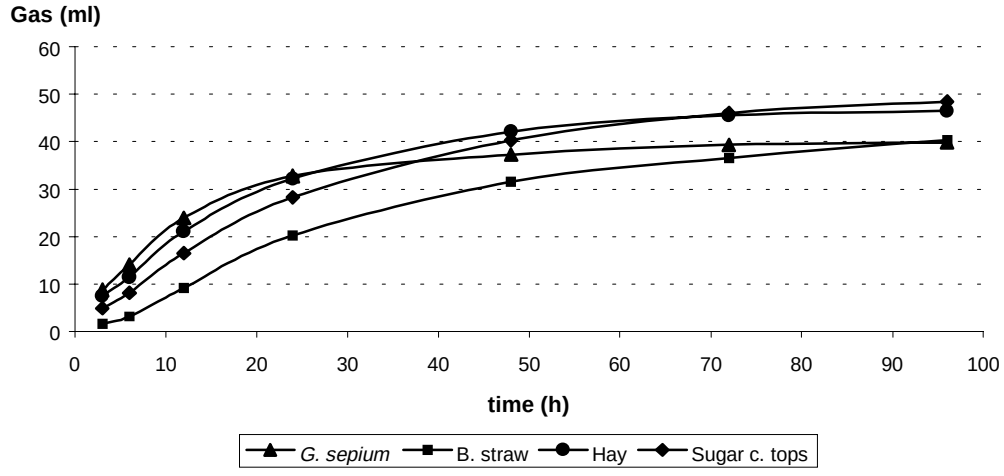
4.1- Forages characterisation.

As part of the initial characterisation of the forages used, table 1 shows the chemical composition of the original samples. Figure 1 and table 2 show the *in vitro* gas production and Figure 2 and table 3 the DM *in sacco* degradability, respectively. Data shows that nutritive value of forage is indeed different, as measured by chemical analysis also based on the gas production and rumen degradability analysis.

Table 1. Chemical composition of the forages (% DM basis).

Forage	*DM (%)*	*Ash*	*Total N*	*NDF*	*Total sugars*
Gliricidia	94.6	6.8	3.24	42.1	28.05
Barley straw	89.3	3.3	0.55	86.9	62.21
Hay	87.4	5.6	1.07	73.0	54.12
Sugar c. tops	93.1	3.6	0.78	79.2	58.47

Fig. 1-*In vitro* gas production of the original forages



Analysing the characteristic of both gas production and rumen degradability it can be seen that the highest cumulative gas production was in sugar cane tops and hay, whereas Gliricidia and straw were lowest. It can be seen also that Gliricidia had the greatest (P<0.001) degradation rate and rumen degradability/gas production at 48h. The lower values in those two parameters (P < 0.001) were obtained from the straw and the sugar cane tops.

Table 2- *In vitro* gas production values of the original forages.

	ml gas at 48h		C value(% h-1)	
	X	SD	X	SD
Gliricidia	37.30^{b}	0.34	0.0732^{d}	0.0032
Barley straw	31.50^{a}	0.91	0.0278^{a}	0.0010
Hay	42.10^{d}	0.69	0.0469^{c}	0.0011
Sugar cane tops	40.30^{c}	0.69	0.0341^{b}	0.0008

Letters indicate significant differences (P < 0.001)

Fig. 2- *In sacco* degradability of the original forages (raw data)

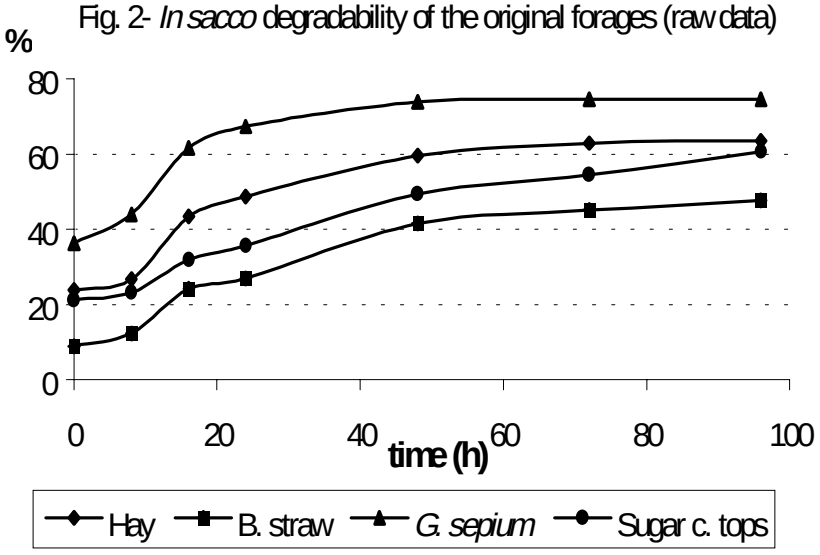


Table 3- *In sacco* degradability values of the original forages.

	%degrad. 48h		C value (%)	
	X	SD	X	SD
Gliricidia	73.86[d]	0.34	0.0997[d]	0.0030
Barley straw	41.60[a]	0.91	0.0366[b]	0.0015
Hay	59.60[c]	0.69	0.0623[c]	0.0013
Sugar cane tops	49.36[b]	0.69	0.0221[a]	0.0001

Letters indicate significant differences ($P < 0.001$)

4.2- Washing loss from nylon bag and filter paper methods.

The results of washing losses in each forage, both *in sacco* and filter paper, are shown in Figure3. It can be see that the *in sacco* washing losses were higher than the filter paper. This result was expected due to the losses of both ready soluble material and small particles that leave the bag through the pores. In addition, some soil particles could contribute, as part of the soluble fraction of the sample. The differences between forages could be due to the individual characteristic of each ones, mainly species and maturity. Blümmel *et al*., (1996) evaluated the electrical energy required to reduce by grinding a defined quantity of straw as a variable to assess its nutritive values. Bowman and Firkins (1996) analysing gamagrass, orchardgrass and red clover found differences between forages grinding it with two-mesh size. The presence of more ready water-soluble material and/or the quantity of the small particles escaping the bag can be responsible for that.

Fig. 3- Influence of the separation procedure on washing losses.

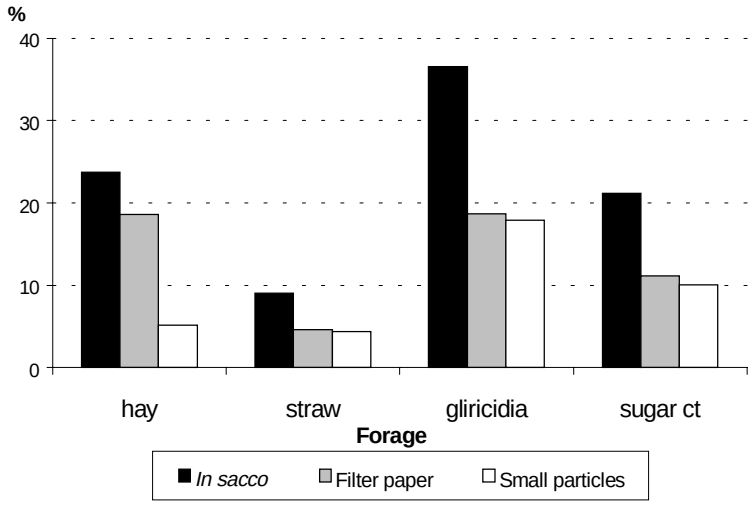


Fig. 4-Influence of the sample washing on ash contents.

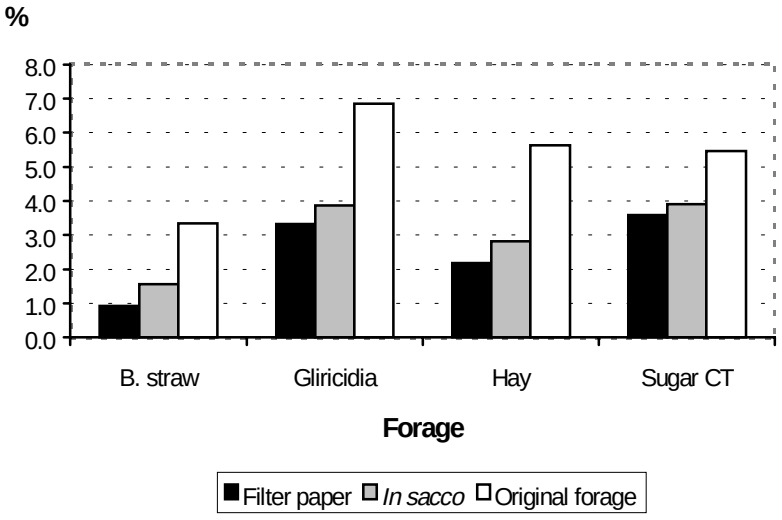
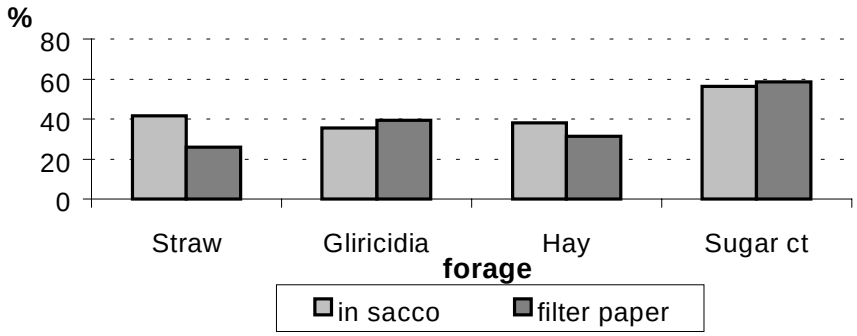


Fig 4a -Ash concentration in the soluble fraction.



Even if the samples were milled in the same mill the differences in small particle proportion will probably depend on the vegetal structure of the forages, *i.e*. degree of lignification and fragility. It is well know that differences between forages in small particles occur during the milling process, even in the same equipment and screen size. Pike *et al*., (1996) analysing treated and untreated Barley straw using the *in sacco* technique suggested that the physical form of the material used should be standardised. It is know that fineness of grinding affects the washing losses of the material. Nevertheless, it does not appear to affect the rumen DM degradation curve characteristics (Kyle and Hovell, 1987). The small particles produced by grinding dried samples may result in an overestimation of zero time losses (López *et al*., 1995).

The quantity of small particles is larger too in Gliricidia and smallest in straw. Proportionally the hay has the lowest losses in small particles if it is compared with the rest of the forages. It is assumed that the small particles that leaves the nylon bag will be digested, as well as the soluble fraction (Ørskov, 1992).

Figure 4 shown the influence of the sample washing on the ash contents. It was noted

that all forages loose minerals. This leaching effect removed great quantity of mineral, both soluble and associated to the small particles. It was represented a great proportion of minerals in the soluble fractions (Figure 4a). Flachowsky and Grun (1992) and Flachowsky *et al.*, (1994) also found losses of minerals after *in sacco* washing of Italian ryegrass and untreated and ammonia treated wheat straw.

The washing procedure also altered the NDF contents in the insoluble fractions, Figure 5 show the NDF values in the residues and the original material. It was observed that the Gliricidia foliage had the greatest changes. It can be seen in the other forages that when the filter paper procedure was used the NDF values are near to 100% of residues. These indicate a possibility of study the use of such a procedure as a simple and clean way to determine NDF in some forages.

Figure 6 represents the sugar contents, determined from the hydrolysis of total carbohydrates, and the changes related with the washing procedure in all forages. All forages follows the same trend, the filter paper procedure remove more no-sugar-related material than the *in sacco* method. The straw reached the highest value of sugar and Gliricidia the lowest one. In general, the presence of more soluble materials in the *in sacco* residue shows that the such a washing procedure did not removed all the water-soluble substances in any of the study forages.

Fig. 5- Influence of sample washing on NDF content

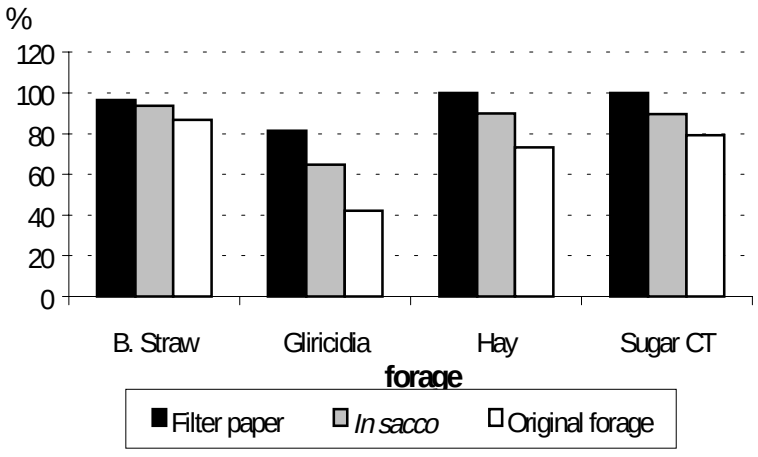
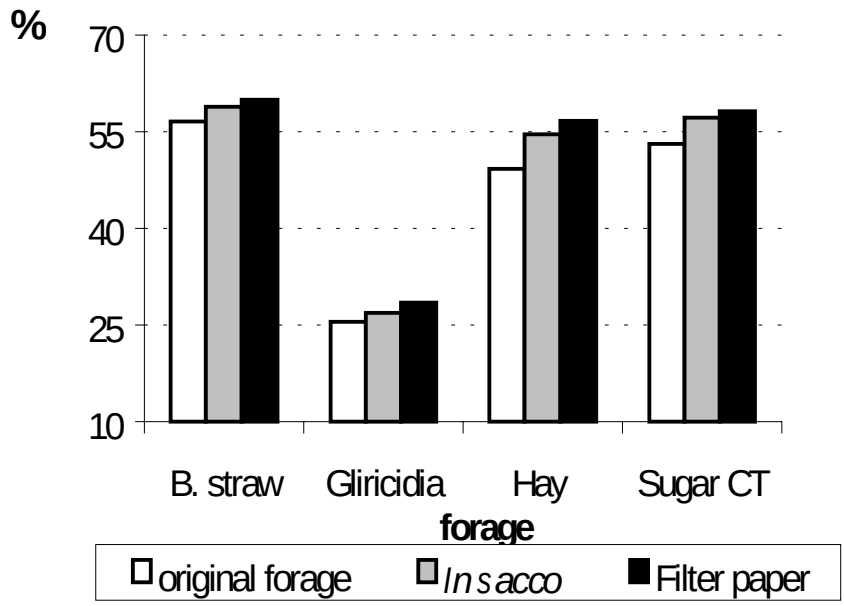


Fig. 6- Influence of the sample washing on total sugars content.



4.3- *In vitro* gas production from the insoluble fractions.

From Figures 7 to 10, and tables 3 to 7, it is show the *in vitro* gas production from both the original forages and its different fractions. *In vitro* gas production from Gliricidia fractions (Figure 7 and table 3) show that the insoluble residues had a lower ($P < 0.001$) 48h gas production and b, a+b and c values than the original forage. The great losses of soluble materials leave the insoluble fraction with an evident low nutritive value. It would appear that the insoluble material of Gliricidia was less fermentable than from straw (table 4 and Figure 8). The suggestion by Pathirana and Ørskov (1995) that one contribution of Gliricidia was supply of easy fermentable fibre to straw look seems doubtful.

Fig 7. Influence of the washing procedure on the gas production of *G. sepium*.

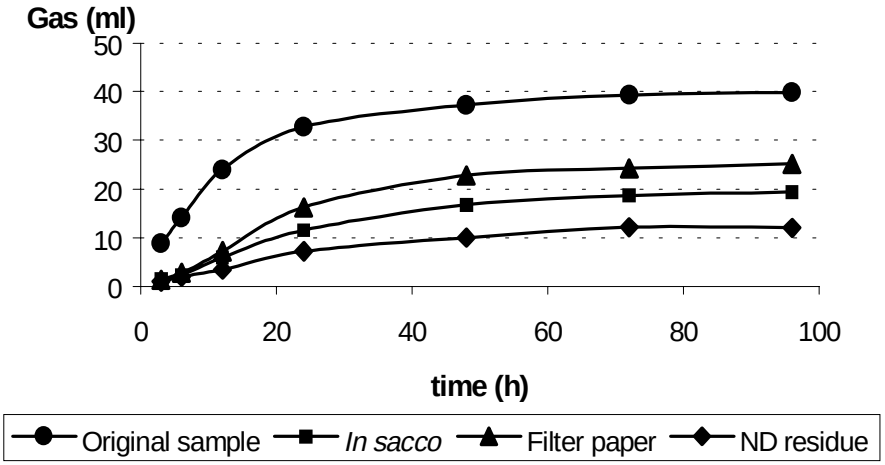
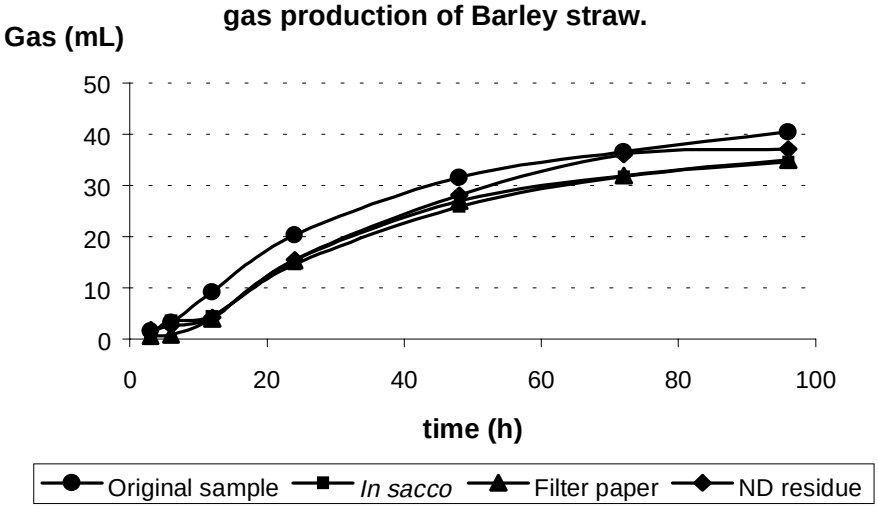


Fig. 8 - Influence of the washing procedure on the gas production of Barley straw.



The gas production from NDF reach the lowest ($P < 0.001$) values of 48h gas production to b and a+b fraction. It can be seen noted that in some test, carried out at IFRU, Rowett Research Institute. Aberdeen, UK, was not found effect on gas production due to the possibility of detergent remaining in the sample. Nevertheless the NDF solution could remove some no soluble compounds from the sample, like pectin, that can make contribution on the *in vitro* gas production. For all that reasons, there could be two explanation for low gas production from the insoluble residues, 1) the greater concentration of any antimicrobial substances in the insoluble residues, and/or 2) Gliricidia insoluble fractions do not make an important contribution of easy fermentable fibre.

Table 4 – Influence of the washing procedure on gas production characteristics of *G. sepium*.

	ml gas at 48h		b (ml)		a+b (ml)		C value(% h-1)	
	X	SD	X	SD	X	SD	X	SD
Original forage	37.30^{d}	0.34	38.36^{d}	0.97	39.34^{d}	0.54	0.0733^{c}	0.0030
In sacco	16.70^{b}	0.01	21.56^{b}	0.40	20.15^{b}	0.07	0.0370^{ab}	0.0007
Filter paper	22.80^{c}	0.94	29.20^{c}	1.17	26.02^{c}	1.13	0.0427^{b}	0.0064
Neutral deterg.	9.93^{a}	0.30	13.37^{a}	0.81	12.90^{a}	0.49	0.0330^{a}	0.0010

Letters indicate significant differences ($P < 0.001$)

In vitro gas production features of the barley straw and its insoluble residues are shown in Figure 8 and table 5. It was seen that there were not significant differences between the original forage and its insoluble fractions for b value. There were differences ($P < 0.05$) in the 48h gas production and the c value; greatest in the original material, while the insoluble residues gas production were similar. The a+b fraction reached the highest values ($P < 0.05$) in the original material and the neutral detergent insoluble residue, the *in sacco* and filter paper residues were similar in this parameter.

Table 5 – Influence of the washing procedure on gas production characteristics of barley straw.

	ml gas at 48h		b (ml)		a+b (ml)		c value(% h-1)	
	X	SD	X	SD	X	SD	X	SD
Original forage	31.50[b]	0.91	46.59	1.47	43.48[b]	0.99	0.0267[b]	0.0015
In sacco	25.85[a]	0.35	43.98	0.94	41.66[a]	0.38	0.0200[a]	0.0008
Filter paper	26.90[a]	1.73	44.63	0.53	40.32[a]	0.72	0.0233[a]	0.0032
Neutral deterg.	28.13[a]	1.15	48.70	2.93	45.65[b]	2.93	0.0203[a]	0.0015

Letters express significant differences ($P < 0.01$) for gas at 48h and ($P < 0.05$) for the rest.

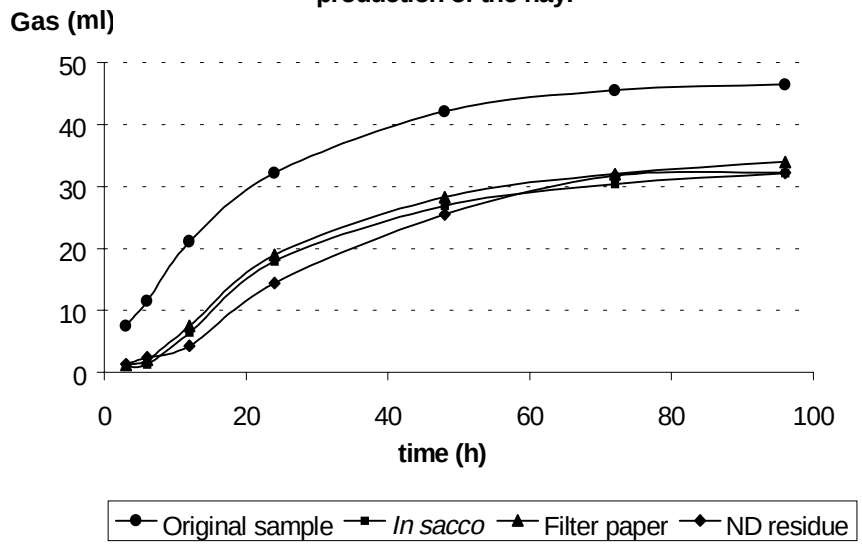
Table 6 and Figure 9 show the gas production characteristics of hay and its insoluble residues. It was observed that the original material reached the highest values ($P < 0.01$) of gas production at 48h, b and a+b fraction, while the insoluble residues had similar value. The c value was also highest in the original material, while the *in sacco* and filter paper residues had the greatest values ($P < 0.001$) compared with neutral detergent insoluble residue.

Table 6 – Influence of the washing procedure on gas production characteristics of hay.

	ml gas at 48h		b (ml)		*a+b (ml)*		c value(% h-1)	
	X	SD	X	SD	X	SD	X	SD
Original forage	42.10[b]	0.69	46.06[b]	0.51	47.04[b]	1.04	0.0470[c]	0.0010
In sacco	26.85[a]	0.91	38.58[a]	0.07	34.06[a]	0.38	0.0335[b]	0.0035
Filter paper	28.27[a]	1.10	40.04[a]	0.66	35.87[a]	0.17	0.0332[b]	0.0033
Neutral deterg.	25.50[a]	1.05	41.14[a]	1.99	38.10[a]	2.08	0.0233[a]	0.0005

Letters indicate significant differences ($P < 0.001$)

Fig 9 -Influence of the washing procedure on the gas production of the hay.



The influence of the washing procedure on gas production characteristics of sugar cane tops and the soluble residues are shows in table 7 and Figure 10. It is seen that the original material reach the greatest ($P < 0.01$) values of gas production at 48h and c value. The b value was similar in both the original material and the insoluble residues, while a+b fraction was highest ($P < 0.05$) in neutral detergent residues; the original material and filter paper residues reach similar values. The a+b value in *in sacco* insoluble fraction was the lowest ($P < 0.05$). It is probably that the hot detergent treatment could improves the quality of the insoluble residue in sugar cane tops, if so this effect was not noted in the rest of forages.

In general, the closed differences in gas production between the original forage and the insoluble residues is observed in the straw while in the others forages such a difference were more notorious, mainly in Gliricidia. Also it was noted a mayor lag phase in the insoluble fractions when it is compared with the original sample, effect obviously due to the lack of ready available organic matter to the rumen microbes.

Table 7 – Influence of the washing procedure on gas production characteristics of sugar cane tops.

	ml gas at 48h		b (ml)		*a+b (ml)*		c value(% h-1)	
	X	SD	X	SD	X	SD	X	SD
Original forage	40.30[d]	0.69	50.96	1.16	50.37[a]	1.06	0.0343[c]	0.0005
In sacco	26.60[b]	0.00	48.22	0.00	45.19[b]	0.00	0.0215[b]	0.0021
Filter paper	29.40[c]	0.58	52.58	2.42	48.98[a]	2.20	0.0202[b]	0.0013
Neutral deterg.	21.13[a]	0.49	57.87	6.67	57.09[c]	6.78	0.0100[a]	0.0010

Letters indicate significant differences (P < 0.001)

Fig 10- Influence of the washing procedure on the gas production of Sugar cane tops.

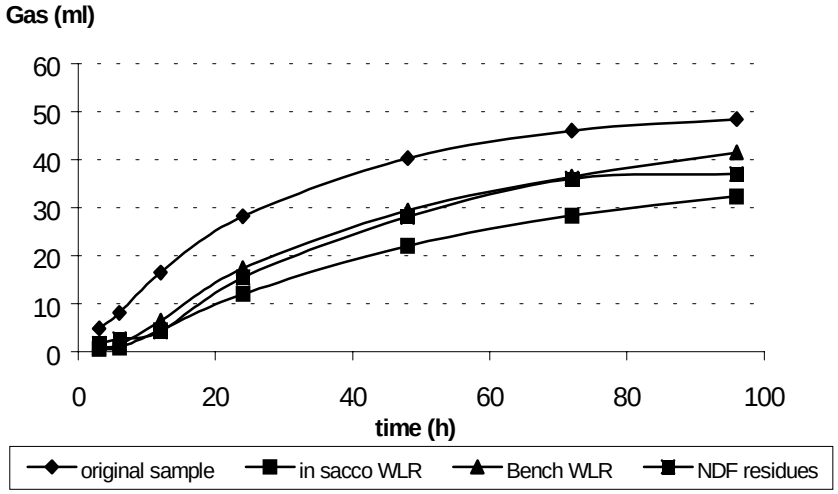
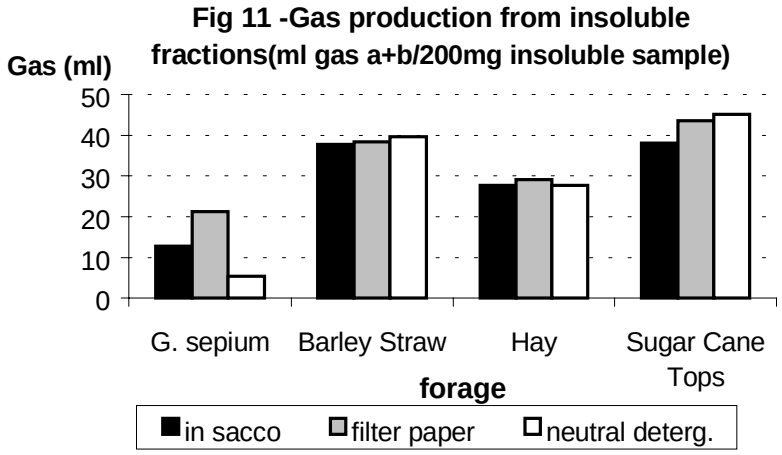


Figure 11 corroborates the behaviour of gas production from the different insoluble fractions. It was noted that Gliricidia had lower *in vitro* gas production per unit of insoluble sample than the rest, while sugar cane tops reached the highest value in this ratio. It is know that the cell wall contend of Gliricidia is associated to lignin and cutin (Escobar *et al*., 1996), nevertheles it still considered a source of easily fermented fibre. Jung and Allen (1995) described negative effect on digestion of the cell wall associated compound, like phenolic acids and the cross-linkage of lignin and wall

polysaccharides. The slight differences between insoluble fractions it is also seen, this point out the necessity of standardisation of the separation methods.

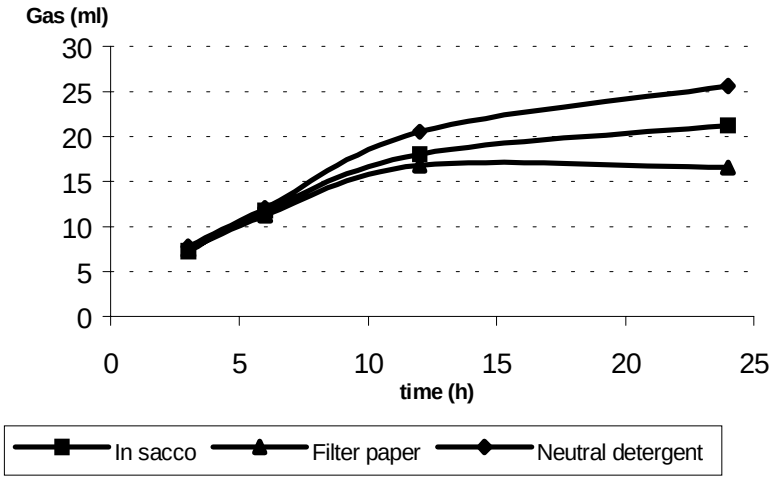


4.4 – *In vitro gas* production from the soluble fractions.

From Figure 11 to Figure 14, it is show the *in vitro* gas production from the soluble fractions. Figures 11, 12, 13 and 14 reflects the effect of the separation methods on the individual soluble fractions from Gliricidia, barley straw, hay and sugar cane tops respectively.

The gas productions from the Gliricidia soluble fractions have reached the greatest values while the straw has the lowest one. It was noted that in the three Gliricidia fractions the gas production reached the plateau over 12 h. This show that the soluble fraction in this legume is degraded and/or release slowly from small particles, which as an important effect in ruminants nutrition.

Fig 12- Contribution of the soluble fractions of *G. sepium*



Probably the benefits effect attributed to its contribution in high digestible fibre it is due the fact of slowly release of its soluble fraction. Nevertheless, it is also probable that, *in vivo* condition, this fraction can reach the small intestine as undegraded substrate. This procedure needs obviously more attention and future research. Stefanon *et al.*, (1996) also found differences between forages, alfalfa and brome hay, in the gas production contribution from the soluble fractions and the rate of digestion of this fraction. Schofield and Pell (1995) studying the degradation of the neutral detergent-soluble fraction in grasses and legumes found the presence of faster-digesting material in legumes.

The soluble fraction from barley straw made a low contribution to gas production, in general the plateau of gas production was reached at 12 h, or probably before. Hay has a similar behaviour but with greater gas production than straw. The sugar cane tops followed the same behaviour than Gliricidia, in general reach the plateau after 12 hour but with about the 50% gas contribution when it is compared with Gliricidia.

It was expected that a great mineral content in this fraction could affected the quantity of available organic matter to the rumen microbes, but there is necessary more data for an adequate correlation analysis. Coincidentally this effect is very clear in straw that shows a very low gas production and a great mineral content in its soluble fractions. Other factor that can contribute to the low gas production in this soluble fraction could be the presence of soluble but not digestible material.

Fig 13- Contribution of the soluble fractions of Barley straw.

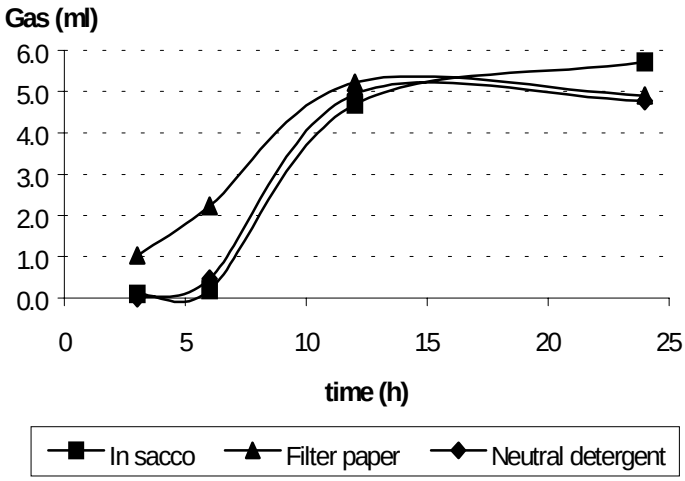


Fig 14 -Contribution of the soluble fractions of hay.

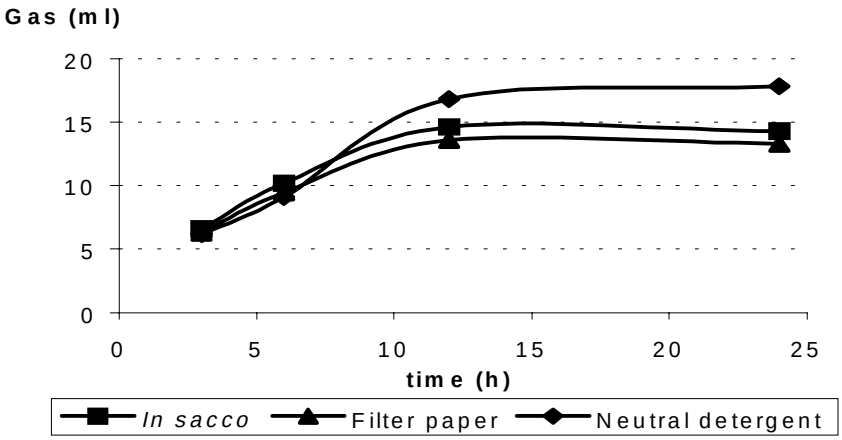
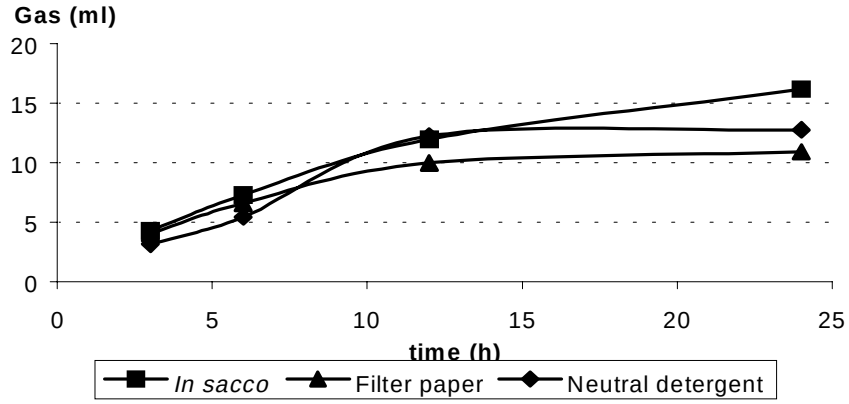
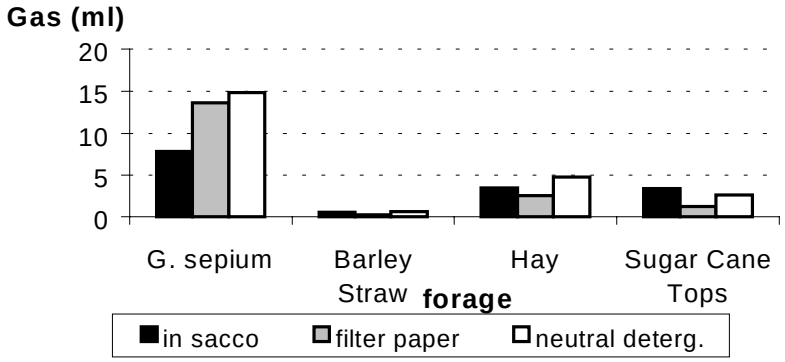


Fig 15 -Contribution of the soluble fraction of Sugar cane tops.



In Figure 16 it is seen the gas production at plateau per unit of soluble sample. The differences between forage are clearly noted, even amount different methods of sample separation. Gliricidia shows the highest gas production and straw the lowest. Hay and sugar cane tops are intermediate. In this Figure and in Figure 11 it is seen the differences between methods of separation.

Fig 16- Gas production from soluble fractions (ml at plateau/200mg soluble sample)



5.0 - Conclusions

All washed samples showed losses of soluble material, and also small particles in the nylon bag washing method. In general Gliricidia had the greatest washing loss and straw the least. The *in vitro* gas production was in general significantly higher for the unwashed forages. Gliricidia gave the highest values of gas production and straw the lowest. The rate of gas production in the insoluble fraction between methods was similar in *in sacco* and filter paper, while detergent solution tended to be lower. The gas production of the insoluble Gliricidia fraction was lower than the other forages, while it reached the highest values from the fermentation of its soluble fraction. Such data suggests that the contribution of the fibrous material on the nutritive value of Gliridicia may be limited. Therefore, the role of Gliricidia in supplying readily fermentable fibre and improve rumen function should be studied in more detail. The results indicated differences between separation methods. It was noted some negative influence of high ash concentration in the soluble fraction on *in vitro* gas production. This influence seen to be greatest on forages with small washing losses. The results suggested that it is generally more appropriate to measure the degradation of organic matter as usual dry matter can give problems of interpretation of the significance of the soluble fraction.

6.0 – References

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