*Rev. prod. anim.*, 25 (1): 2013

# Validation of Ovine Excreta Using *in vitro* Gas to Assess Feed for Ruminants

*Mileidys González Rodríguez, Alex A. Resillez Pujal, Redimio M. Pedraza Olivera y Silvio J. Martínez Sáez*

*** Faculty of Agricultural Sciences, University of Camagüey, Cuba**

**misleidis.gonzalez@redu.edu.cu**

## ABSTRACT

Ovine excreta as inoculum for biogasification was used to assess *in vitro* and *in sacco* nutritive value of tropical pastures for ruminants. Pastures under study were: *Paspalum notatum, Cynodon nlemfuensis, Panicum maximum, Sporobolus indicus, and Desmodium* sp. Excreta were collected on grassy pastures areas. $CO_2$ systematic injections prevented strict anaerobic microorganisms contact with oxygen. A positive and significant correlation resulted from biogasification using either ovine excreta or ovine ruminal fluid. Determinant coefficient was 0,79. *Panicum maximum* and *Cynodon nlemfuensis* reached the highest nutritive value, while *Sporobolus indicus* showed the lowest one.
**Key Words**: *ovine excreta, digestibility, nutritive value*

## INTRODUCTION

Ruminants are an important source of food, as they turn products (forages and legumes) into high quality food like milk and meat, a process humans and other single-stomach organisms cannot do (Ørskov, 1999; Panin, 2000; Moore y Jung, 2001; Preston, 2003; FAO, 2008).

Food evaluation methods have been developed to serve several purposes, including animal yielding improvements (France *et al*., 2000).

Some of the nutritional assessing methods used nowadays are *in situ* or *in sacco* ruminant degradability, and *in vitro* gas production.

The use of feces as inoculum to perform *in vitro* feed evaluation leads to similar reports by other authors, using rumen fluid, thus preventing stress and animal physical damage in the process of drawing the rumen fluid (Akhter y Hossain, 1998; Mauricio *et al*., 2001; Van Thu, 2003).

Research done by Martínez (2005), Hernández (2006) and Resillez (2008) in the province of Camagüey clearly show the potential of bovine droppings as inoculum to carry out nutritional assessment of forages, using *in vitro* gas production. However, higher ruminants cause more expenses in terms of maintenance as lab animals. Hence, lower ruminants, especially ovine, are used. The aim of this paper is to validate ovine excreta using *in vitro* production of gas to assess ruminant feed.

## MATERIALS AND METHODS

*Experiment 1. Dynamics of* in vitro *gas production with ovine rumen fluid*

Several pasture samples were analyzed: Texan *(Paspalum notatum), Star (Cynodon nlemfuensis), Guinea (Panicum mazimum), Straw (Sporobolus indicus)* and *Crawling legume (Desmodium* sp.*)*. Some dried samples (200 mg) were put in syringes, and carefully shaken when heated in warm bath at 39 °C . A siphon was used to draw rumen fluid from the fistulate animals. The dilution was used with softened mineral medium (1 + 2) and the samples were incubated by triplicate. The general procedure used to produce gas *in vitro* stems from the principles by Menke *et al.* (1979), using 100 ml glass syringes (1 ml minimum). Readings were done every two hours for the first 24 h; then every 24 h, until 96 h .

*Experiment 2. Dynamics of* in vitro *gas production from ovine excreta, using dilution 1 + 3*

The samples were analyzed just like in the previous experiment, only the inoculum was changed. The ovine excreta for the inoculum were collected within the first three hours of dropped, indoors, where all animals sleep, and early in the morning, as suggested by Akhter *et al.* (1996). Then they were mixed with the softened mineral medium (1 + 3), in home blender for about a minute. The solution was filtered through inert sieve material (Dederon), in order to remove the solid particles (Martínez, 2005).

The procedure was done by systematic injection of $CO_2$ to keep oxygen from only anaerobic microorganisms.

The procedure used for *in vitro* gas production was the same as before. According to Pedraza (1998), the procedure was adjusted to modifica-

tions in LABCA conditions (Martínez, 2005). A volume of 30 ml of the inoculum was added to each syringe and the samples were incubated by triplicate. The gas volume was measured every three hours during the first 24 h and at 24, 48 and 72 h after the run.

## RESULTS AND DISCUSSION

The *in vitro* production of gas with rumen fluid is already established (Ramachandra and Krishnamoorthy, 2000; Van Thu, 2003).

Figure 1 shows the highest production of gas reaching up to 25 ml and more for legumes from 24 hours on. Straw pasture (*Sporobolus indicus*) has less nutritional value tan star (*Cynodon nlemfuensis*), guinea (*Panicum maximum*), and Texan (*Paspalum notatum*). The similarity of forage behavior for both methods is apparent. The curves are monotones for the *in vitro* production of gas with rumen fluid.

Different behavior in the dynamics for the *in vitro* production of gas among the inocula from several animals corroborates observations by (Mertens *et al.*, 1997; Rymer *et al.*, 1999; Nagadi *et al.*, 2000; Cone *et al.*, 2002; Tscherning *et al.*, 2002), reporting that the offspring may be related to the characteristics of the animal diet. It has a significant influence on the rumen´s microbial composition. Some variations may be observed for similar animals, even with inoculum prepared identically (Bueno *et al.*, 2005).

The substrate that goes to the large intestine is different from the one that enters the rumen, since most easily-digested nutrients have been removed and, also, because other endogenous materials have added, like mucopolysaccharides and enzymes. During microbial digestion of the large intestine volatile fatty acids are produced and then absorbed. Gases are also produced, but in general terms, digestion in the intestine is less efficient than in the rumen (McDonald *et al.*, 2002).

Figure 2 shows that dynamic behavior for the *in vitro* production of gas will depend, like every microbial growth process, on the substrate, the medium and the inoculum (Menke *et al.*, 1979; Mauricio *et al.*, 2001; Martínez, 2005). The volume of gas produced by *in vitro* incubation of a substrate is closely related to its digestibility and; therefore, with its energy value (Menke and Steingass, 1988; Getachew *et al.*, 2004)

The little difference in the production of gas during the first hours owes to the necessary adjustment phase of microorganisms to the new substrate (Menke *et al.*, 1979; Mauricio *et al.*, 2001). When adjustment is over, the gas production dynamics will basically depend on the amount of acid produced by microorganisms from the source of carbon supplied.

In that case, it is like in Figure 1, in relation to the production of gas from pastures; that is, straw (*Sporobolus indicus)*, with the lowest volume of gas. All the other pastures produce more than 5 ml of gas after 48 h . The graphic with droppings tends to be sigmoidal.

When excreta is used the volume of gas produced after 72 h it is lower than when rumen fluid is used, which is the expected behavior (Mauricio *et al.*,2001; Cone *et al.*, 2002; Martínez, 2005; Martínez, 2008).

An explanation for lower productions of gas using excreta given by Mould *et al.* (2005) claims that fecal micro flora has hydrolytic profiles different from those of rumen. These authors suggest that there are more qualitative (metabolic) than quantitative differences among the inocula. Cone *et al.* (2002) reported similar conclusions. All this may be influenced by the animal diet, both in composition and under processing. In this work only animals fed with poor-quality and quantity pastures. Tract microorganisms, closely associated with wastes from rumen, are also excreted with feed residues in the excreta (Theodorou *et al.*, 1994). The fecal matter basically remains anaerobic after dropped and the micro flora is alive for several hours (Holter, 1991).

The cultures inoculated with fecal matter need more time to achieve degradation potential than the ones using rumen fluid, but when they reach certain threshold, the growing speed is similar in both cases. Dhanoa *et al.* (2004), suggest the addition of more microorganisms is inoperative when the maximum degradation speed is reached.

In the excreta, microorganisms are under “suspended animation”, due to the lower number of substrates: they try to survive and their metabolic activity is decreased. However, the microorganisms in the rumen, growing in a substrate-rich medium have a different behavior (Cone *et al.*, 2002).

Figure 3 shows the positive correlation among the volumes of cumulated gas when rumen´s fluid

and ovine droppings are used, thus strengthening the idea that the latter might be used as inoculum (Martínez, 2008).

Aiple *et al*. (1992) described a modification in the method of Tilley and Terry (1963), by using an ovine excreta (feces) suspension as inoculum to produce *in vitro* digestion by gas production. Later on, Akhter *et al*. (1999) in comparative works between bovine feces and ovine rumen fluid concluded that the droppings may be an alternative to evaluate *in vitro* digestibility of forages.

## CONCLUSIONS

Ovine feces may be used as inoculum to produce gases for *in vitro* and *in sacco* nutritional assessments of forages for ruminant feed.

Positive and significant correlations were observed between the production of gas with ovine feces and using ovine rumen samples. The dynamics for *in vitro* production of gas using ovine rumen fluid and ovine droppings indicate that the forages assessed are arranged from higher to lower nutritional value.

## REFERENCES


AIPLE, H.; STEINGASS y MENKE, K. H. (1992). Suitability of a Buffered Fecal Suspension as The Inoculum in the Hohenheim Gastes. *Anim. Nutr., 67*, 57-66.

AKHTER, S. y HOSSAIN, M. M. (1998). Cow Faeces *in vitro* Digestibility Assays of Forages. *Asian-Australasian Journal of Animal Sciences*, *11* (1), 51-54.

AKHTER, S.; OWEN, E. y HOSSAIN, M. M. (1996). Use of Cow Faeces at Different Times after Being Voided as a Source of Micro-Organisms *in vitro* Digestibility Assays of Forages. *Asian-Australasian Journal of Animal Sciences, 9* (4), 371-374.

AKHTER, S.; OWEN, E.; THEODOROU, M. K.; BUTLER, E. A. y MINSON, D. J. (1999). Bovine Faeces as a Source of Micro-Organisms for the *in vitro* Digestibility Assay of Forages. *Blackwell Science Ltd, Grass and Forage Science, 54*, 219-226.

BUENO, I. C.; CABRAL FILHO, S. L.; GOBBOA, S. P.; LOUVANDINI, H.; VITTI, D. M y ABDALLA, A. L. (2005). Influence of Inoculum Source in a Gas Production Method. *Animal Feed Science and Technology*, 123-124 y 95-105.

CONE, J. W.; VAN GELDER, A. H. y BACHMANN, H. (2002). Influence of Inoculum Source on Gas Production Profiles. *Anim. Feed Sci. Technol., 99*, 221-231.

DHANOA, M. S.; FRANCE, J.; CROMPTON, L. A.; MAURICIO, R. M.; KEBREAB, E.; MILLS, J. A. N.; SANDERSON, R.; DIJKSTRA, J. y LÓPEZ, S. (2004). Technical Note: A Proposed Method to Determine the Extent of Degradation of a Feed in the Rumen from the Degradation Profile obtained with the *in vitro* Gas Production Technique Using Feces as the Inoculum. *J. Anim. Sci*., 82, 746.

FAO. (2008). *Ayudando a desarrollar una ganadería sustentable en Latinoamérica y el caribe: lecciones a partir de casos exitosos*. Chile, Santiago de Chile: Oficina Regional para América Latina y el Caribe. Organización de las Naciones Unidas para la Agricultura y la Alimentación.

FRANCE, J.; THEODOROU, M. K.; LOWMAN, R. S. y BEEVER, D. E. (2000). Feed Evaluation for Animal Production. En Theodorou, M. K. y France, J. (Eds.). *Feeding Systems and Feed Evaluation Models.* Wallingford, UK: CAB International.

GETACHEW, G.; DEPETERS, E. y ROBINSON, P. (2004). *In vitro* Gas Production Provides Effective Method for Assessing Ruminant Feeds. *California Agriculture, Jan-Mar*. Extraído en febrero 2009, desde http://www.bioparametrics.com/Pdf/Neil%20Jessop%20Publications.pdf.

HERNÁNDEZ, J. E. (2006). *Valoración de la caprinocultura en la mixteca poblana: socioeconomía y recursos arbóreo-arbustivos*. Tesis de doctorado en Ciencias Veterinarias, Universidad de Camagüey, Cuba.

HOLTER, P. (1991). Concentration of Oxygen, Carbon-Dioxide and Methane in the Air within Dung Pats. *Pedobiología, 35*, 381-386.

MARTÍNEZ, S. J. (2005). *Implementación de la técnica de producción de gas* in vitro *con heces vacunas como inóculo y su empleo para evaluar el follaje de algunas leguminosas arbustivas*. Tesis de maestría en Producción Bovina Sostenible, Facultad de Ciencias Agropecuarias, Universidad de Camagüey, Cuba.

MARTÍNEZ, S. J. (2008). *Heces vacunas depuestas como inóculo en la técnica de producción de gases para la valoración nutritiva* in vitro *de forrajes*. Tesis de doctorado en Ciencias Veterinarias, Universidad de Camagüey, Cuba.

MAURICIO, R. M.; OWEN, E.; MOULD, F. L.; GIVENS, D. I.; THEODOROU, M. K.; FRANCE, D.; DAVIES R. y DHANOA, M. S. (2001). Comparison of Bovine Rumen Liquor and Bovine Faeces as Inoculum for an *in vitro* Gas Production Technique for Evaluating Forages. *Anim. Feed Sci. Technol, 89*, 33-48.

MCDONALD, P.; EDWARDS, R. A.; GREENHALGH, J. F. D. y MORGAN, C. A. (2002). Evaluation of Foods. Digestibility. En *Animal Nutrition* (Sixth Edition). Pearson. Prentice-Hall.

MENKE, K. H. y STEINGASS, H. (1988). Estimation of the Energetic Feed Value Obtained from Chemical Analysis and *in vitro* Gas Production Using Rumen

Fluid. *Animal Research and Development*, *28*, 7-55.

MENKE, K. H.; RAAB, L.; SALEWSKI, A.; STEINGASS, H.; FRITZ, D. y SCHNEIDER, W. (1979). The Estimation of the Digestibility and Metabolizable Energy Content of Ruminant Feedingstuffs from the Gas Production when they are Incubated with Rumen Liquor *in vitro*. *J. agric. Sci*, *93*, 217-222.

MERTENS, D. R.; WEIMER, P. J. y WAGHORN G. C. (1997). Inocula Differences Affect *in vitro* Gas Production Kinetics. *Research Summaries*. Extraído el 16 de agosto de 2008, desde www.dfrc.wisc.edu/RS97pdfs/ RM2.pdf.

MOORE, K. J. y JUNG, H. J. G. (2001). Lignin and Fiber Digestion. *Journal of Range Management, 54*, 420-429.

MOULD, F. L.; MORGAN, R.; KLIEM, K. E. y KRYSTALLIDOU, E. (2005). A Review and Simplification of the *in vitro* Incubation Medium. *Animal Feed Science and Technology, 123-124*, pp. 155-172.

NAGADI, S.; HERRERO, M. y JESSOP, N. S. (2000). The Influence of Diet of the Donor Animal on the Initial Bacterial Concentration of Ruminal Fluid and *in vitro* Gas Production Degradability Parameters. *Anim. Feed Sci. Technol., 87*, 231-239.

ØRSKOV, E. R. (1999). *Nutrición de rumiantes*. Curso de Posgrado. Universidad de Camagüey, Cuba.

PANIN, A. (2000). A Comparative Economic Analysis of Smallholder Cattle and Small Ruminant Production Systems in Botswana. *Tropical Animal Health and Production, 32* (3), 189-196.

PEDRAZA, R. M. (1998). *Use of* in vitro *Gas Production Technique to Assess the Contribution of Both Soluble and Insoluble Fractions on the Nutritive Value of Forages*. MSc thesis, University of Aberdeen, Scotland.

PRESTON, T. R. (2003). *Producción Agropecuaria Sostenible: crisis u oportunidad*. Taller Internacional: Ganadería, desarrollo sostenible y medio ambiente, marzo, La Habana.

RAMACHANDRA, B. y KRISHNAMOORTHY, U. (2000). Evaluation of Some Feeds by *in vitro* Gas Production. *Indian Veterinary Journal, 77* (11), 976-978.

RESILLEZ, P. A. (2008). *Producción de gases con heces vacunas depuestas como inóculo para predecir la degradabilidad* in situ *de forrajes*. Tesis de maestría en Producción Bovina Sostenible, Universidad de Camagüey, Cuba.

RYMER, C.; HUNTINGTON, J. A. y GIVENS, D. I. (1999). Effects of Inoculum Preparation Method and Concentration, Method of Inoculation and Pre-Soaking the Substrate on the Gas Production Profile of High Temperature Dried Grass. *Anim. Feed Sci. Technol*., 78, 199-213.

THEODOROU, M. K.; WILLIAMS, B. A.; DHANOA, M. S.; MCALLAN, A. B. y FRANCE, J. (1994). A Simple Gas Production Method Using a Pressure Transducer to Determine the Fermentation Kinetics of Ruminant Feeds. *Animal Feed Science and Technology, 48*, 185-197.

TILLEY, J. M. A. y TERRY, R. A. (1963). A Two Stage Technique for the *in vitro* Digestion of Forage Crops. *Journal of the British Grassland Society*, *18*, 104-111.

TSCHERNING, K.; BARRIOS, E.; LASCANO, C.; PETERS, M. y SCHULTZE-KRAFT, R. (2002). *Comparison of Aerobic and Anaerobic Methods to Assess Quality of Tropical Multipurpose Shrub Legumes*. Extraído el 10 de agosto de 2009, desde http://mars.wiz.uni-kassel.de/tropentag/proceedings/2002/htmL/node153.

VAN THU, N. (2003). Effect of Different Strategies of Treated Rice Straw on Nutrients and *in vitro* OM Digestibility by Using Rumen Fluid or Faecal Inocula of Local Cattle; En Reg Preston y Brian Ogle (Eds). *Proceedings of Final National Seminar-Workshop on Sustainable Livestock Production on Local Feed Resources*. Hue City: HUAF-SAREC. Extraído en abril de 2009,desde http://www.mekarn.org/sarec03/thu2.htm

VAN THU, N. (2003). Inoculum from Sheep. *Animal Feed Science and Technology*, 123-124, 81-94.

Recibido: 3-9-2012
Aceptado: 3-10-2012

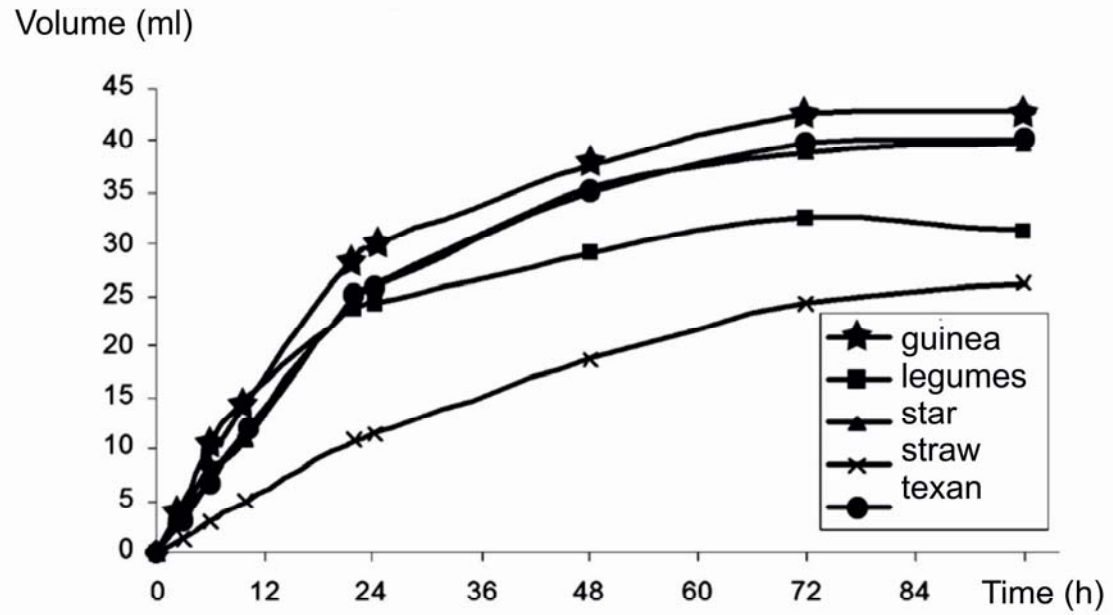



Fig. 1. Production of gas at different times using ovine rumen fluid as inoculum

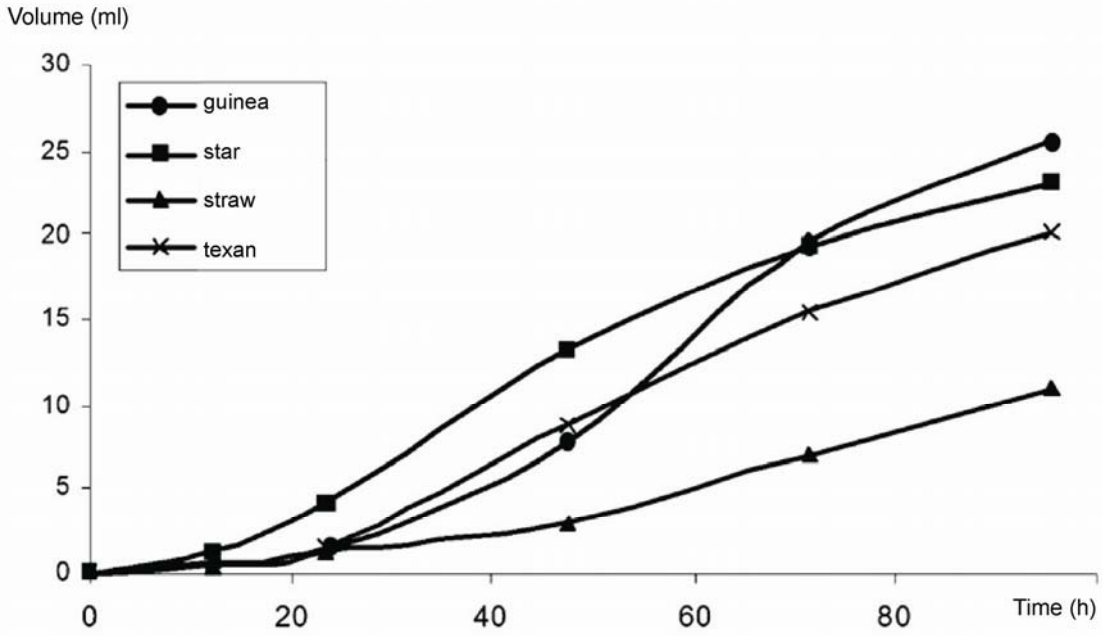
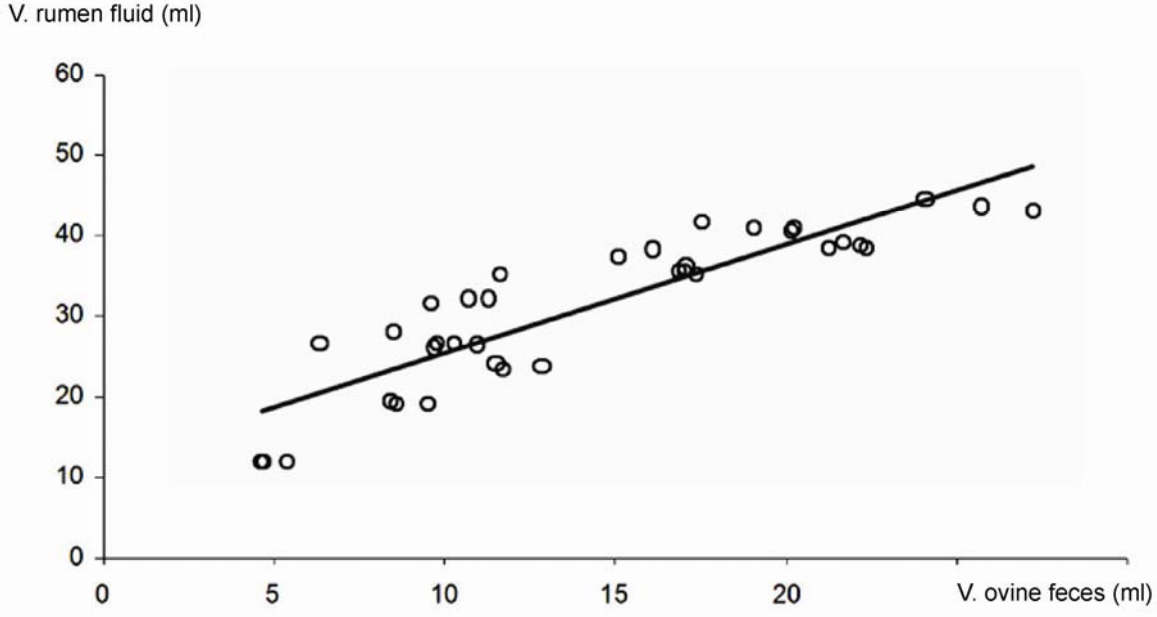



**Fig. 2. Production of gas at different times using ovine feces as inoculum**




$n = 36$; $r^2 = 0{,}78$; $P < 0{,}01$

**Fig. 3. Relation between the volume of gas using ovine feces and using ovine rumen fluid**