***In vitro* digestibility and fermentation kinetics of some browse plants using sheep or goat ruminal fluid as the source of inoculum**

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**Abstract**

To explore the hypothesis that different ruminant species may differ in their ability to digest browse forages in the rumen, two *in vitro* experiments were conducted using batch cultures inoculated with rumen fluid (RF) obtained from either sheep or goats fed on the same diet (alfalfa hay). *In vitro* dry matter digestibility (IVD) and gas production kinetics were determined for twenty-four samples of leaves, flowers and fruits of five browse plant species: *Erica australis; Cistus laurifolius;* *Quercus pyrenaica; C. scoparius* and *Rosa canina,* collected at upland sited in the province of León (NW Spain) from spring to autumn. There were no IVD differences between sheep and goat RF for any of the browse plant samples. The extent of degradation in the rumen was higher (*P* < 0.05) with goat RF for leaves and flowers of *R. canina* and young leaves and fruits of *Q. pyrenaica*, with higher values (*P* < 0.05) with sheep RF for leaves of *C. laurifolius* harvested in September. Likewise, dry matter disappearance at 144 h was generally higher with goat RF and differences (*P* < 0.05) were mainly detected for leaves and flowers of *R. canina.* However, although statistical differences between both sources of inoculum were not consistent among and within plant species, asymptotic gas production and gas production at 24 h were generally higher with sheep RF. Gas production rate was faster (*P* < 0.05) with sheep RF only for young leaves of *E. australis* and *C. scoparius* and leaves of *C. laurifolius* collected in June and September; however, with goat RF higher (*P* < 0.05) values were detected for flowers of *C. laurifolius*, fruits and mature leaves of *Q. pyrenaica* and flowers and young leaves of *R. canina*. Although some interspecies differences in the *in vitro* ruminal fermentative activity were detected in the present study, it can be concluded that when animals are fed the same diet, differences between sheep and goat rumen fluid used as source of inoculum can be considered of little nutritional significance.

*Keywords:* Sheep; Goat; *In vitro* digestibility; Gas production; Shrub

*Abbreviations:* IVD, *in vitro* digestibility; dg, extent of degradation; D144, dry matter disappearance after 144h of incubation; A, asymptotic gas production; G24, cumulative gas production at 24h; c, fractional rate of gas production; RF, rumen fluid.

**1. Introduction**

In some extensive ruminant production systems, browse plants may represent an important source of nutrients to grazing animals. Many of these plants contain relatively high levels of condensed tannins. Therefore, whether by choice or necessity, herbivores consume tannins and these secondary compounds can have detrimental or beneficial effects on animal nutrition (Min *et al.*, 2003; Waghorn and McNabb, 2003). Potential benefits include protein sparing in the rumen and anthelminthic effects, whereas some unfavourable effects are microbial inhibition and decreased feed digestibility and animal performance. Given their potentially adverse effects on animal nutrition, these compounds have been considered as antinutritional factors. The concentration of tannins in some feedstuffs (forages, shrubs, tree foliage) and their effects on digestive utilization of feeds have been studied extensively, but there is less available information about the susceptibility of different ruminant species to the effects of the tannins on efficiency of digestion.

Based on their ability to browse shrub and tree foliage, ruminant species have been classified as grazers or browsers (Gordon, 2003). Within the same group, animal response to tanniniferous diets depends largely on its physiological capacity to adapt to high tannin levels in the diet. Although the digestive tract of goats is anatomically similar to that of sheep, studies conducted under field conditions and *in vivo* revealed that goats are more efficient than sheep digesting feedstuffs with low nitrogen, high fibre or high tannin contents (El Hag, 1976; Reid *et al.*, 1990; Masson *et al.*, 1991; Tolkamp and Brouwer, 1993). In these *in vivo* trials it is not possible to differentiate if differences between both species can be attributed to a different digesting activity of microbial population in the rumen, given the multiplicity of factors that will affect the total tract digestibility, such as ability of selection of diet on offer, chewing/eating behaviour, gut physiology, digestive compartment dimensions or digesta passage rate (Van Soest, 1994; Mould *et al.*, 2005). *In vitro* assays using rumen fluid from each ruminant species to inoculate the cultures may be a useful tool to examine possible differences in the digesting capacity of the microbial population in the rumen. These possible differences are also important to decide if a given ruminant species (sheep) can be used as a model to estimate *in vitro* digestibility of some conventional feeds. Although there are some comparative studies in the *in vitro* digestibility of forages using sheep and cattle rumen fluid as sources of inoculum, very few comparisons between sheep and goat have been reported up to date. This study was conducted to detect interspecies differences between sheep and goats based on *in vitro* incubations using rumen fluid from both ruminant species as inoculum to determine *in vitro* digestibility and kinetics of production of fermentation gas**.**

**2. Material and methods**

*2.1. Source of shrubby samples*

Twenty-four samples of leaves, flowers and fruits (pods) from five browse species, namely *Erica australis* L. (Spanish heath), *Cistus laurifolius* L. (laurel-leaved rock-rose), *Quercus pyrenaica* Willd (hoary oak), *Cytisus scoparius* (L.) Link (Scotch broom) and *Rosa canina* L. (wild dog rose) were collected in 1998. The selection of the species was based on the available information on preference and intake by sheep and goats, and on their relative abundance in the area of study, the uplands of the province of León (Northwest of Spain). Samples were taken at different times from early spring (leaf flushing) to late autumn (leaf fall of deciduous species), at the sampling times specified in Table 1, so plants were at different maturity stages. The sampling area was situated at an altitude of 900 m above sea level, with an average annual rainfall and temperature of 564 mm and 10.6ºC, respectively. Branches and twigs of several specimens of each species were clipped with scissors and immediately taken to the laboratory, where leaves, flowers and fruits (when available) were manually separated. Then samples were immediately freeze-dried and milled in a hammer mill using a 1 mm sieve. Chemical composition and tannin content of the samples (and methods for chemical analysis) has been reported elsewhere, and a brief account of this information is provided in Table 1 (Ammar *et al.*, 2004a, 2004b, 2004c).

*2.2. Animals and extraction of rumen fluid*

Rumen fluid was withdrawn before the morning feed from four adult Merino sheep and four Alpine goats fitted with permanent rumen cannulae. Animals of both species were housed and maintained under similar conditions; all received the same feed (1 kg/d of good quality alfalfa hay) and were sampled identically. Samples of rumen contents of animals from the same species were combined and collected separately into thermos flasks, and taken immediately to the laboratory. Rumen fluid of each animal species was strained through four layers of cheesecloth, and kept at 39ºC under a CO2 atmosphere.

*2.3. In vitro digestibility*

The procedure followed was the *in vitro* filter bag method in ANKOM Daisy incubators (Ammar *et al.*, 1999). Samples (250 mg) were weighed into ANKOM F57 polyester/polyethylene bags (size 5 cm × 5 cm; pore size 25 μm), which were sealed with a heater and placed in incubation jars. Each jar was a 5-L glass recipient with a plastic lid provided with a single-way valve which avoids the accumulation of fermentation gases. For each source of inoculum (sheep or goat rumen fluid) two incubation jars were used and 25 bags were placed in each jar (one for each sample plus one empty bag). Buffered rumen fluid (RF) was prepared according to Ammar *et al.* (1999) diluting fresh RF in the culture medium (200 mL RF/L and 800 mL medium/L) at 39º C and under anaerobic atmosphere. A special “complete” medium was formulated to provide any essential factor for microbial growth, with the aim that none of the microorganisms existing in the rumen contents of sheep or goats could undergo any growth inhibition or limitation for a deficit of a nutrient or growth factor. Thus, the basal medium of Menke and Steingass (1988) containing buffer, macro-mineral, micro-mineral, resazurin and reductive solutions was enriched with trypticase, yeast, haemin, branched-chain fatty acids, coenzyme M and a mixture of sheep and goat clarified RF (Table 2). For each incubation run, fresh RF from sheep and goats was collected the same day and at the same time. Each buffered RF was prepared separately but at the same time. Under anaerobic conditions, 2 L of each buffered RF were transferred into the jars (two with sheep RF and two with goat RF) containing the bags. The jars were then placed in a revolving incubator (ANKOM Daisy) at 39ºC, with continuous rotation to facilitate the effective immersion of the bags in the rumen fluid. After 48 h of incubation in buffered RF, bags were gently rinsed under cold tap water, and then rinsed again in a washing machine (short 10-min washing cycle with cold water). Bags were dried at 60ºC for 48 h and then washed out in a neutral detergent solution at 100ºC for 1 h. The dry residue was weighed out and considered as the truly indigestible matter to calculate the *in vitro* DM digestibility (IVD). Incubations were performed in two runs carried out in two consecutive weeks (four replicates of each feed sample per each source of inoculum).

##### *2.4. In vitro gas production*

##### The method used for gas production measurements was as described by Theodorou *et al.* (1994). About 500 mg of each sample were weighed out into 120 mL serum bottles pre-warmed at 39ºC and flushed with CO2. Four bottles were used for each substrate in each incubation run (two bottles for each inoculum source). Blanks without substrate were incubated in each run and for each inoculum. Fifty ml of buffered RF prepared as described before were anaerobically dispensed in each bottle at 39°C. The culture medium used was the same enriched medium described above. All the bottles were crimped with aluminium caps and placed in the incubator at 39ºC, being shaken at regular times. Volume of gas produced in each bottle was recorded at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h after inoculation time, using a pressure transducer (Theodorou *et al.*, 1994). At the end of the incubation period, the contents of each serum bottle were filtered under vacuum using sintered glass crucibles and oven-dried at 100°C for 48 h to calculate the potential DM disappearance (D144, g/g DM). The exponential model proposed by France *et al.* (2000) was fitted to gas production profiles:

##### , where

#####  *G* (mL/g) denotes the cumulative gas production at time *t*; *A* (mL/g) is the asymptotic gas production; *c* (/h) is the fractional rate of gas production and *L* (h) is the lag time. According to France *et al.* (2000), the extent of degradation in the rumen (*dg*, g/g DM) for a given rate of passage (*k*, /h) can be estimated as . In order to calculate *dg*, a rate of passage of 0.03 /h (characteristic for sheep and goats fed a forage diet at maintenance level) was used. Incubations were performed in two runs carried out in two consecutive weeks (four replicates of each feed sample per each source of inoculum).

*2.5. Statistical analysis*

One-way analysis of variance (Steel and Torrie, 1980) was carried out with *in vitro* dry matter digestibility (IVD) data and gas production parameters of all studied samples to examine the differences between sheep and goat RF. In the statistical model, the incubation run was considered as a blocking factor, and the source of inoculum (sheep vs. goat RF) as the only treatment factor. The statistical significance of the differences between means was evaluated using the least significant difference test. Simple linear correlation analysis (Steel and Torrie, 1980) was used to establish the relationship between *in vitro* digestibility and gas production kinetics determined either with sheep or with goat RF across all the browse samples.

##### 3. Results

For the entire group of shrub species studied herein, IVD was not affected (*P* > 0.05) by the source of RF (Table 3). Regardless the source of RF, the lowest values corresponded to flowers of *E. australis* and the highest to flowers of *C. scoparius*. Asymptotic gas production (A), cumulative gas produced after 24 h of incubation (G24) and fractional rate of gas production (c) for the different browse plant species incubated in sheep and goat RF are shown in Table 4, and average coefficients of *in vitro* DM disappearance after 144h of incubation (D144) and of extent of degradation (dg) in the rumen are in Table 5. Although the effect of inoculum source on G24 and A was not consistent among and within plant species, there was a tendency for increased values with sheep RF, but values were different (*P* > 0.05) with sheep or goat inocula only for foliage of *E. australis*, fruits of *C. laurifolius* and leaves (collected in May), flowers and fruits of *C. scoparius*. Fermentation rates (c values) were higher when sheep RF was used to incubate young leaves of *E. australis* and *C. scoparius* and leaves of *C. laurifolius* collected in June and September. When cultures were inoculated with goat RF, fermentation rates of flowers of *C. laurifolius*, fruits and mature leaves of *Q. pyrenaica* and flowers and young leaves of *R. canina* were increased.

##### The *dg* values revealed some differences (*P* < 0.05) between RF of sheep and goats. Extent of degradation of leaves of *C. laurifolius* harvested in September was greater in sheep RF, whereas degradability of leaves and flowers of *R. canina* and young leaves and fruits of *Q. pyrenaica* was greater with goat RF (Table 5)*.* There were also differences (*P* < 0.05) in D144 of some browse samples between sheep and goat inocula. Values were higher when sheep RF was used for leaves *of E. australis* (August) and flowers of *C. scoparius*, whereas D144 was greater (*P* < 0.05)with goat RF for young leaves of *C. laurifolius* and *Q. pyrenaica*, flowers of *E. australis*, young and mature leaves and flowers of *R. canina.*

In spite of the source of inoculum, IVD and *in vitro* gas production parameters showed the highest values with foliage of *C. scoparius* and the lowest with *E. australis.* Moreover, the parameters from leaves of the different shrubs showed, generally, a progressive decrease from spring to autumn. A drastic decrease was observed with goat RF, especially when leaves of *Q. pyrenaica* were incubated.

##### 4. Discussion

It is well established that ruminants can tolerate higher levels of antinutritional factors in feeds than monogastric animals, because ruminal bacteria can metabolize some toxic compounds, such as tannins. Amongst ruminant species, it has been reported that goats are better adapted than sheep to tolerate rich-tannin diets (Provenza *et al.*, 1990). In the present study, incubation of plant material in sheep or goat RF had no effect (*P* > 0.05) on IVD (Table 3), even with shrubs such as *E. australis* characterized by low crude protein and high tannin contents (Ammar *et al.*, 2004a, 2004b). IVD determined with either sheep and goat RF were highly correlated (r = 0.996; n = 24; *P* < 0.001), with a regression coefficient close to unity (slope = 0.966 ± 0.0285). These results are in agreement with those reported by other authors (Molina Alcaide *et al.*, 1997, 2003; Jones *et al.*, 2001; Gordon *et al.*, 2002) and could be due in part to a possible extraction of condensed tannin contents of the samples by neutral detergent solution that can minimize the interspecies differences. Jones *et al.* (2001) observed no differences in the *in vitro* DM digestibility of various shrub legumes rich in condensed tannins when ruminal fluid obtained from different South African wild browser ruminant species and from sheep was used to measure IVD**.** These authors concluded that it seemed unlikely that these ruminant species had rumen bacteria capable of degrading condensed tannins contained in these shrub legumes. Digestion of such tanniniferous feeds may well depend on tannin binding with proline-rich saliva proteins rather than on metabolism of or tolerance to condensed tannins by rumen bacteria.

##### The *in vitro* gas production technique has received much attention as a means of evaluating the nutritional quality of feedstuffs (Williams, 2000). In the present study, the main value of this technique was to detect differences between fermentative activity in rumen fluid of sheep and goats when shrubs with different tannin contents were incubated. This technique is considered more sensitive to detect such differences than other *in vitro* gravimetric techniques (Williams, 2000). Inhibition of growth of the predominant rumen bacteria by polyphenolics has been proved *in vitro* in pure cultures, and this effect could affect the production of fermentation gas when plant material are incubated in cultures of mixed ruminal microorganims (Rymer *et al.*, 2005).

##### *A* and *G24* values tended to be higher when browse foliage was incubated in buffered sheep RF, especially with low nutritional quality browse species (*E. australis, C. laurifolius and Q. pyrenaica*) (Ammar *et al.*, 2004 a,b). These observations would indicate a higher fermentative activity in sheep RF compared with goat RF. However, these possible differences were not so noticeable when values of extent of degradation in the rumen (*dg*) were compared. The gas production technique has been used to compare fermentative activity of sheep and cattle (Cone *et al.*, 2002; Bueno *et al.*, 2005), or of sheep and buffalo (Calabrò *et al.*, 2005). In these studies all animals received the same diet, and it was observed that differences between sources of inoculum in volumes of gas produced were small, with a close correlation between values recorded with sheep and cattle rumen fluid. However, fermentation rates estimated for the gas production kinetics with rumen fluid from sheep and cattle were not so well correlated (Cone *et al.*, 2002). Calabrò *et al.* (2005) observed that volumes of gas produced at early and late incubation times were similar with sheep and buffalo RF, and some differences between both source of inoculum were observed at intermediate incubation times, especially when more fibrous feeds were fermented. In spite of the occasional differences observed herein between both sources of inoculum for some browse plant species, values of *G24* and *dg* observed with sheep and goat RF were highly correlated (r values of 0.979 and 0.982, respectively; *P* < 0.001) across the whole set of samples studied (n = 24). Fractional fermentation rates (c values) showed also a good relationship (r = 0.946, *P* < 0.001).

##### Most studies reported in the literature have concluded that whereas differences between sheep and goats in total tract digestibility and rumen degradability are negligible for medium-high quality forages, roughages with high fibre and low nitrogen contents are digested to a greater extent in goats than in sheep (Jones *et al.*, 1972; El Hag, 1976; Brown and Johnson, 1984; Reid *et al.*, 1990; Masson *et al.*, 1991; Tolkamp and Brouwer, 1993; Isac *et al.*, 1994; Molina Alcaide *et al.*, 1997, 2000). Thus, it has been suggested that goats would be superior than sheep digesting forage cell wall (Domingue *et al.*, 1991a), probably due to higher counts of cellulolytic bacteria (Gihad *et al.*, 1980; Tisserand *et al.*, 1991), higher enzyme activity to hydrolyze structural polysaccharides (Gado, 1999) or more favourable and stable conditions in the rumen for microbial activity (Hadjipanayiotou and Antoniou, 1983; González López *et al.*, 1990).

##### Goats could be also more efficient than sheep digesting rich-tannin feedstuffs (Degen *et al.*, 1997; Odenyo *et al.*, 1999), as a result of a tolerance to these antinutritional factors, attributed to the presence in the rumen of goats of bacterial species (*Streptococcus caprinus*) resistant to the detrimental action of condensed tannins (Brooker *et al.*, 1994) or the possible production of an active tannase enzyme (Begovic *et al.*, 1978). Narjisse *et al.* (1995) observed that sheep were more sensitive than goats to the ruminal infusion of tannins obtained from *Q. ilex*. However, Odenyo and Osuji (1998) could not find differences between cultures inoculated with either sheep or rumen fluid in the sensitivity to tannins and Pérez-Maldonado and Norton (1996) did not observe differences between both ruminant species in the digestive utilization of various tropical roughages with high tannin contents. In the present experiment differences between both inocula were always small, and did not seem to be related to the tannin content of the plant material, probably because the chemical nature and structure of tannins, and not only their concentration in the plant, may affect their effects on ruminal microorganisms and on substrate degradation in the rumen (Hagerman *et al.*, 1992). In general, with both sources of inoculum feedstuffs used in this study were ranked similarly, so that it may be expected that differences among browse plant species, and between plant parts or sampling times within each species are not affected by the source of rumen fluid (sheep or goat) used in the incubations.

##### Nevertheless, some of the differences observed *in vivo* between sheep and goats in the digestive utilization of forages can be attributed to differences in ingestive behaviour (patterns of eating, chewing and rumination), rumen size and fill, digesta passage kinetics (Domingue *et al.*, 1991a, b; Kennedy *et al.*, 1992; Tisserand *et al.*, 1991). All these effects would be attenuated and minimized *in vitro*, thus reducing the differences between both species. On the other hand, comparative studies between ruminant species are highly determined by the diet fed to the animals, so that differences become more noticeable when animals of each species are fed a different diet, and are considerably reduced when all animals receive the same diet (Mould *et al.*, 2005). Goats are also more selective than sheep, and thus when animals have free access to forages or browse, goats will be more efficient in selecting the most digestible fractions of the feed on offer (García *et al.*, 1995; Papachristou, 1997). In the present study all donor animals were confined in individual cages and fed the same forage, medium-high quality hay with which it is not necessary that animals develop special strategies to digest substrates with high fibre and low nitrogen concentrations. It is commonly accepted that microbial population of animals (even of different species) housed in closed contact and offered similar feeds will tend to uniformity (Mould *et al.*, 2005). Feeding level was relatively low so that animals had little opportunity to select their diet from the feed on offer, thus minimizing differences in selective behaviour between animals which might account for a different digestive efficiency. Finally, given the type of forage used, it is not expected that animals may develop special adaptive mechanisms in response to the consumption of antinutritive compounds (tannins). Based on other results reported in the literature (Brown and Johnson, 1984; Reid *et al.*, 1990; Domingue *et al.*, 1991b; Masson *et al.*, 1991; Tolkamp and Brouwer, 1993; Isac *et al.*, 1994; Molina Alcaide *et al.*, 1997), it can be speculated that differences in in *vitro* digestibility when sheep or goat RF are used as source of inoculum could be larger if animals were fed a poor quality roughage (high in fibre and tannins and low in nitrogen).

Our results would be in agreement with theories suggesting that inter-species variations may be the result of animal-plant interactions resulting in adaptive changes in response to the type of diet selected by each animal species, that would have a major effect on the type, concentration and activity of ruminal microorganisms and thus on the extent of degradation of feeds in the rumen (Gordon, 2003). Differences observed among ruminant species in substrate digestion efficiency would be a consequence of a dietary adaptation rather than real species-specific and intrinsic differences (Gordon *et al.*, 2002).

##### 5. Conclusion

Differences between sheep and goat rumen fluid used as source of inoculum of cultures of mixed ruminal microorganisms to determine in vitro digestibility and fermentation kinetics can be considered of little nutritional significance based on results reported herein. In most cases differences between both sources of inoculum were not significant, with a few cases in which estimations of fermentation rate or of extent of degradation were different between both ruminant species (for some feedstuffs higher with sheep, and for others higher in goat rumen fluid). Differences in in vitro digestibility among the different browse plant species and effects of stage of maturity of each plant species on digestibility were similar regardless the source of inoculum used. The fact that all donor animals were fed the same diet and were maintained under the same conditions may explain the lack of relevant differences between both animal species.

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**Table 1**. Chemical composition (g/kg dry matter) of leaves, flowers and fruits of the browse species (Ammar *et al.*, 2004a, 2004b, 2004c)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Plant species | Plant part | Sampling time | Organic matter | Crude protein | Neutral detergent fibre | Lignin | Extractable tannins\* | Condensed tannins† |
| *Erica australis* | Leaves | May | 976 | 52 | 387 | 164 | 112 | 816 |
| August | 979 | 56 | 405 | 217 | 115 | 746 |
| October | 979 | 52 | 393 | 185 | 121 | 951 |
| Flowers | April | 979 | 65 | 407 | 249 | 64 | 648 |
| *Cistus laurifolius* | Leaves | June | 957 | 134 | 409 | 80 | 130 | 257 |
| September | 955 | 76 | 291 | 65 | 73 | 331 |
| November | 959 | 72 | 256 | 67 | 54 | 242 |
| Flowers  | June | 966 | 85 | 276 | 37 | 120 | 14 |
| Fruits  | July | 968 | 73 | 485 | 95 | 141 | 248 |
| *Quercus pyrenaica* | Leaves  | June | 957 | 163 | 462 | 62 | 93 | 11 |
| August | 946 | 118 | 500 | 101 | 88 | 130 |
| November | 940 | 65 | 573 | 165 | 51 | 93 |
| Flowers  | June | 949 | 130 | 410 | 98 | 77 | 17 |
| Fruits  | October | 980 | 60 | 445 | 36 | 39 | 55 |
| *Cytisus scoparius* | Leaves  | May | 944 | 277 | 220 | 28 | 29 | 5 |
| July | 946 | 221 | 280 | 53 | 26 | 5 |
| August | 940 | 164 | 299 | 58 | 23 | 7 |
| Flowers  | May | 956 | 216 | 257 | 58 | 11 | 6 |
| Fruits | June | 963 | 181 | 393 | 33 | 13 | 4 |
| *Rosa canina* | Leaves  | May | 934 | 176 | 340 | 28 | 107 | 42 |
| July | 927 | 127 | 419 | 30 | 113 | 107 |
| November | 917 | 59 | 322 | 53 | 127 | 100 |
| Flowers | June | 948 | 94 | 311 | 60 | 111 | 184 |
| Fruits | September | 957 | 78 | 441 | 125 | 20 | 122 |

\* Extractable tannins (as phenols precipitable in polyvinylpyrrolidone (g tannic acid equivalent/kg dry matter)

† Condensed tannins measured with the butanol-HCl assay (g quebracho equivalent/kg dry matter)

**Table 2**. Composition of the enriched culture medium used in the *in vitro* incubations

|  |  |
| --- | --- |
| Ingredient | per L of medium |
| Distilled water | 375 mL |
| Buffer solution § | 237 mL |
| Macromineral solution § | 237 mL |
| Micromineral solution § | 1.25 mL |
| Trypticase | 1.25 g |
| Yeast extract | 0.625 g |
| Haemin solution (1 g haemin/L) | 1.25 mL |
| Coenzyme M solution (40 g CoM/L) | 1.25 mL |
| Branched-chain VFA + S solution(0.30 M isobutyric acid, 0.70 M valeric acid,0.90 M isovaleric acid, 0.94 M Na2SO3) | 2.15 mL |
| Clarified ruminal fluid †(mixture in equal parts of sheep and goat rumen fluid) | 94 mL |
| Resazurin solution § | 1.10 mL |
| Reducing solution § | 50 mL |

§ These solutions were prepared according to Menke and Steingass (1988)

† Prepared according to Leedle and Hespell (1980)

**Table 3**. Effect of source of inoculum (sheep or goat) on *in vitro* dry matter digestibility (g digested/g incubated) of leaves, flowers and fruits of some browse species

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Plant species | Plant part | Sampling time | Rumen Fluid | SED |
| Sheep | Goat |  |
| *Erica*  | Leaves | May | 0.659 | 0.643 | 0.0157 |
| *australis* | August | 0.591 | 0.586 | 0.0080 |
|  | Oct | 0.600 | 0.598 | 0.0063 |
|  | Flowers | April  | 0.541 | 0.539 | 0.0160 |
| *Cistus*  | Leaves | June  | 0.706 | 0.699 | 0.0073 |
| *laurifolius* | September | 0.718 | 0.708 | 0.0097 |
|  | November  | 0.718 | 0.699 | 0.0191 |
|  | Flowers  | June  | 0.800 | 0.794 | 0.0163 |
|  | Fruits  | July  | 0.569 | 0.570 | 0.0091 |
| *Quercus* | Leaves  | June  | 0.732 | 0.736 | 0.0083 |
| *pyrenaica* | August  | 0.630 | 0.616 | 0.0126 |
|  | November  | 0.591 | 0.581 | 0.0057 |
|  | Flowers  | June  | 0.676 | 0.670 | 0.0170 |
|  | Fruits  | October  | 0.829 | 0.833 | 0.0087 |
| *Cytisus* | Leaves  | May  | 0.855 | 0.831 | 0.0160 |
| *scoparius* |  | July  | 0.865 | 0.869 | 0.0239 |
|  |  | August | 0.856 | 0.853 | 0.0078 |
|  | Flowers  | May | 0.907 | 0.930 | 0.0203 |
|  | Fruits  | June  | 0.757 | 0.738 | 0.0100 |
| *Rosa* | Leaves  | May  | 0.875 | 0.869 | 0.0081 |
| *canina* |  | July  | 0.857 | 0.863 | 0.0064 |
|  |  | November  | 0.843 | 0.856 | 0.0068 |
|  | Flowers | June  | 0.718 | 0.725 | 0.0078 |
|  | Fruits  | September  | 0.612 | 0.610 | 0.0064 |
| Overall comparison |  | 0.729 | 0.726 | 0.0027 |
|  |  | (P=0.177) |

SED: standard error of the difference (n = 4)

**Table 4**. Effect of source of inoculum (sheep or goat) on *in vitro* fermentation kinetics of leaves, flowers and fruits of some browse species

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | *A* (ml/g) |  | *G24* (ml/g) |  | *c* (/h) |  |
| Plant species | Plant part | Sampling time | Sheep | Goat | SED | Sheep | Goat | SED | Sheep | Goat | SED |
| *Erica australis* | Leaves | May | 136a | 125b | 3.6 | 93a | 76b | 5.4 | 0.051a | 0.039b | 0.0027 |
| Aug | 109a | 84b | 4.5 | 73 | 60 | 5.7 | 0.047 | 0.053 | 0.0043 |
| Oct | 116a | 91b | 4.7 | 76a | 59b | 5.2 | 0.047 | 0.044 | 0.0033 |
| Flowers | Apr | 184a | 162b | 2.3 | 140a | 122b | 3.7 | 0.059 | 0.059 | 0.0034 |
| *Cistus laurifolius* | Leaves | June | 145 | 146 | 3.9 | 103a | 87b | 2.3 | 0.052a | 0.037b | 0.0007 |
| Sept | 157 | 147 | 12.5 | 106a | 75b | 10.8 | 0.049a | 0.029b | 0.0024 |
| Nov | 193 | 187 | 6.3 | 134 | 117 | 10.9 | 0.051 | 0.041 | 0.0047 |
| Flowers  | June | 257 | 256 | 10.3 | 236 | 240 | 9.1 | 0.108b | 0.122a | 0.0045 |
| Fruits  | July | 174a | 158b | 2.6 | 134 | 128 | 4.7 | 0.062 | 0.069 | 0.0036 |
| *Quercus pyrenaica* | Leaves  | June | 211b | 218a | 1.9 | 159b | 175a | 3.2 | 0.060b | 0.068a | 0.0014 |
| Aug | 166 | 159 | 3.4 | 110 | 113 | 3.2 | 0.046b | 0.052a | 0.0010 |
| Nov | 138 | 131 | 2.8 | 80 | 81 | 0.9 | 0.036b | 0.040a | 0.0016 |
| Flowers  | June | 175 | 175 | 4.6 | 130 | 134 | 3.6 | 0.058 | 0.061 | 0.0030 |
| Fruits  | Oct | 318b | 326a | 1.0 | 239b | 252a | 3.4 | 0.071b | 0.088a | 0.0028 |
| *Cytisus scoparius* | Leaves  | May | 304a | 285b | 3.2 | 243a | 219b | 3.3 | 0.070a | 0.061b | 0.0021 |
| July | 294 | 279 | 5.9 | 245a | 234b | 2.5 | 0.081 | 0.079 | 0.0024 |
| Aug | 284 | 260 | 10.8 | 250 | 231 | 8.3 | 0.096 | 0.096 | 0.0022 |
| Flowers  | May | 305a | 288b | 2.1 | 279a | 267b | 1.6 | 0.108 | 0.112 | 0.0037 |
| Fruits | June | 322a | 302b | 4.1 | 214 | 195 | 7.6 | 0.048 | 0.044 | 0.0031 |
| *Rosa canina* | Leaves  | May | 273 | 265 | 4.1 | 209 | 215 | 3.4 | 0.063b | 0.072a | 0.0012 |
| July | 266 | 263 | 6.9 | 174 | 185 | 8.1 | 0.049 | 0.052 | 0.0014 |
| Nov | 256 | 257 | 3.5 | 164 | 162 | 1.5 | 0.044 | 0.042 | 0.0014 |
| Flowers | June | 244b | 260a | 5.7 | 186 | 202 | 7.0 | 0.062 | 0.064 | 0.0027 |
| Fruits | Sept | 189 | 193 | 4.3 | 173 | 183 | 4.7 | 0.104b | 0.125a | 0.0032 |
| Overall comparison |  | 217 | 209 | 1.48 | 165 | 158 | 1.63 | 0.064 | 0.064 | 0.0009 |
|  |  | (P<0.001) | (P<0.001) | (P=0.883) |

*A*: asymptotic gas production (ml/g dry matter); *G24*: cumulative gas production at 24 h (ml/g dry matter); c: fractional rate of gas production (/h).

Means within each row and for each variable with different superscripts (a, b) represent different values (*P* < 0.05) for sheep and goat ruminal fluid

SED: standard error of the difference (n = 4)

**Table 5**. Effect of source of inoculum (sheep or goat) on extent of degradation in the rumen of leaves, flowers and fruits of some browse species

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | *D144* (g/g) |  | *dg* (g/g) |  |
| Plant species | Plant part | Sampling time | Sheep | Goat | SED | Sheep | Goat | SED |
| *Erica australis* | Leaves | May | 0.481 | 0.506 | 0.0126 | 0.289 | 0.284 | 0.0117 |
| Aug | 0.433a | 0.410b | 0.0076 | 0.262 | 0.261 | 0.0124 |
| Oct | 0.449 | 0.431 | 0.0078 | 0.262 | 0.255 | 0.0111 |
| Flowers | Apr | 0.427b | 0.447a | 0.0066 | 0.284 | 0.296 | 0.0068 |
| *Cistus laurifolius* | Leaves | June | 0.373b | 0.404a | 0.0105 | 0.235 | 0.224 | 0.0058 |
| Sept | 0.511 | 0.510 | 0.0089 | 0.303a | 0.253b | 0.0099 |
| Nov | 0.538 | 0.545 | 0.0112 | 0.331 | 0.313 | 0.0102 |
| Flowers  | June | 0.752 | 0.781 | 0.0183 | 0.571 | 0.615 | 0.0176 |
| Fruits  | July | 0.447 | 0.429 | 0.0109 | 0.299 | 0.299 | 0.0080 |
| *Quercus pyrenaica* | Leaves  | June | 0.560b | 0.610a | 0.0102 | 0.366b | 0.419a | 0.0087 |
| Aug | 0.495 | 0.480 | 0.0068 | 0.296 | 0.303 | 0.0034 |
| Nov | 0.462 | 0.472 | 0.0202 | 0.251 | 0.269 | 0.0076 |
| Flowers  | June | 0.488 | 0.498 | 0.0127 | 0.317 | 0.333 | 0.0104 |
| Fruits  | Oct | 0.841 | 0.860 | 0.0075 | 0.505b | 0.536a | 0.0085 |
| *Cytisus scoparius* | Leaves  | May | 0.824 | 0.827 | 0.0130 | 0.561 | 0.554 | 0.0102 |
| July | 0.781 | 0.745 | 0.0153 | 0.542 | 0.533 | 0.0141 |
| Aug | 0.683 | 0.682 | 0.0101 | 0.496 | 0.501 | 0.0052 |
| Flowers  | May | 0.912a | 0.884b | 0.0060 | 0.692 | 0.683 | 0.0064 |
| Fruits | June | 0.835 | 0.837 | 0.0178 | 0.494 | 0.488 | 0.0180 |
| *Rosa canina* | Leaves  | May | 0.749b | 0.801a | 0.0161 | 0.491b | 0.551a | 0.0137 |
| July | 0.780 | 0.789 | 0.0077 | 0.450b | 0.487a | 0.0130 |
| Nov | 0.737b | 0.790a | 0.0062 | 0.430b | 0.456a | 0.0084 |
| Flowers | June | 0.622b | 0.676a | 0.0033 | 0.407b | 0.452a | 0.0048 |
| Fruits | Sept | 0.558 | 0.566 | 0.0111 | 0.433 | 0.452 | 0.0079 |
| Overall comparison |  | 0.611 | 0.625 | 0.0036 | 0.397 | 0.409 | 0.0032 |
|  |  |  | (P<0.001) | (P<0.001) |

*D144*: dry matter disappearance after 144h of incubation (g/g); *dg*: extent of degradation (g/g)

Means within each row and for each variable with different superscripts (a, b) represent different values (*P* < 0.05) for sheep and goat ruminal fluid

SED: standard error of the difference (n = 4)