Faecal liquor as alternative microbial inoculum source for *in vitro* (Daisy^{II}) technique to estimate the digestibility of feeds for camels

Laudadio V.[1], Lacalandra G. M.[2], Monaco D.[2], Khorchani T.[3], Hammadi M. [3], Tufarelli V.[1*]

[1]Department of Animal Health and Welfare, University of Bari, Valenzano, Italy;
[2]Department of Animal Production, University of Bari, Valenzano, Italy;
[3]Livestock and Wildlife Laboratory, Arid Regions Institute, Medenine, Tunisia.

Abstract

The objective of this study was to evaluate different sources of faecal liquor as the microbial inoculum source for an *in vitro* (Daisy^{II}, ANKOM®) technique in order to estimate the digestibility of fodder species browsed by camels at pasture in an arid region of Southern Tunisia. The plants represented in the pasture were: *Artemisia campestris* L., *Atriplex halimus* L., *Frankenia thymifolia* Desf., *Imperata cylindrica* L., *Limoniastrum guyonianum*., *Nitraria retusa* (Forssk) Asch, *Reaumuria vermiculata* L., *Salicornia arabica* L., *Salsola tetragona* Del., *Suaeda mollis* (Desf.) Delile and *Zygophyllum album* L. Rumen liquor (RL) was collected from sheep (*n* = 4) and faecal samples were collected from healthy mature sheep (SF, n = 4), goat (GF, n = 4) and camel (CF, n = 4). The Daisy^{II} incubator was used to evaluate the nutrient digestibility of forages using RL as a control and faecal liquor as alternative microbial inoculum sources. Filter bags containing feed samples were added to the four digestion vessels along with their respective inoculum. Filter bags containing samples were incubated for 48 h and dry matter (DM), organic matter (OM), crude protein (CP), neutral and acid detergent fiber (NDF and ADF) digestibility was determined. There was a significant relationship between estimates, indicating that faecal liquor has the potential to be used instead of rumen fluid for estimation of *in vitro* digestibility of forages. It is concluded that the Daisy^{II} incubator is appropriate for the determination of *in vitro* digestibility of nutrients using faecal liquor. These results would indicate that the use of faecal inoculum provides a valid and accurate estimate of feed digestibility for camel.

Keywords: camel, digestibility, faecal liquor, *in vitro* Daisy^{II} technique.

* Corresponding author: Email: v.tufarelli@veterinaria.uniba.it

1. Introduction

Chemical composition of vegetation is crucial, particularly in combination with *in vitro* digestibility, to evaluate the nutritive value of shrub species, which are not know previously. As a consequence, a detailed survey of browse species is important to identify the better shrub species for camels, in terms of nutrient content and digestibility. *In vitro* digestion techniques using rumen liquor as an inoculum (Tilley and Terry, 1963) have proved useful in assessing the relative digestibility of many feeds (Minson, 1990).

The necessity for fistulated animals to provide this inoculum raises a number of practical problems, e.g. surgical facilities, constant care to avoid infections (particularly in tropical countries) and costs associated with the long-term maintenance of these animals (Mauricio et al., 2001). Several studies, reviewed by Omed et al. (2000), have demonstrated faeces to have high potential as an alternative inoculum for *in vitro* digestibility techniques. The successful use of a liquid suspension of faeces from sheep (Vàradyovà et al., 2005), cattle (Jones and Barnes, 1996; Holden, 1999; Mabjeesh et al., 2000) and recently from horses (Lattimer et al., 2007; Murray et al., 2008) to estimate digestibility of a range of feeds have been reported.

The search for better labor efficiency has led to the development of the Daisy^{II} apparatus (ANKOM® Technology Corp., Fairport, NY), which allows simultaneous incubation of different feedstuffs in sealed polyester bags in the same incubation vessel. Results of experiments reported that the use of rumen fluid (Mabjeesh et al., 2000) or faecal liquor inoculum (Lattimer et al., 2007) with a closed-system fermentation apparatus (Daisy^{II} Incubator) yielded valid *in vitro* estimates of DM, NDF and ADF digestibility of forages and grains. Furthermore, this technique allows the estimation of *in vitro* digestibility of a large number of samples simultaneously, in addition to recover the residue of the final prediction for the *in vitro* digestibility of feeds.

Therefore, the objective of this study was to evaluate the *in vitro* digestibility of nutrients from plant species browsed by camels using Daisy^{II} apparatus of ANKOM® comparing rumen fluid and faecal liquor as the inoculum sources.

2. Materials and methods

Samples of 11 plant species were collected during the Spring of 2007 from approximately 500 Ha of halophyte dominant rangelands in Southern Tunisia. These species are considered the dominant components of the diet of the dromedary herd of the Arid Regions Institute of Medenine Tunisia (33°20′ N and 10°29′ E). The dominant species in the pasture were: *Artemisia campestris* L. (AC), *Atriplex halimus* L. (AH), *Frankenia thymifolia* Desf. (FT), *Imperata cylindrica* L. (IC), *Limoniastrum guyonianum* (LG), *Nitraria retusa* (Forssk) Asch (NR), *Reaumuria vermiculata* L. (RV), *Salicornia arabica* L. (SA), *Salsola tetragona* Del. (ST), *Suaeda mollis* (Desf.) Delile (SM) and *Zygophyllum album* L. (ZA). The plants' collection process and their chemical composition is the same as reported by Laudadio et al. (2009) (Table 1).

In vitro fermentation was conducted for 48 h using the Daisy^{II} incubator. The complete unit consisted of 4 incubation vessels with a capacity of 2 L each. Each vessel contained 1.6 L of buffer solution, 400 mL of either rumen liquor or faecal liquor as the inoculum, and 22 nylon bags. Collected plant samples were ground to pass through 1-mm screen and were mixed 1:1. Each of the feeds was digested in duplicate for each source of inoculum. Nylon filter bags (Ankom F57, ANKOM Tech., Fairport, NY) were rinsed in acetone and allowed to air dry before drying at 100°C for 24 h, after which dry bag weight was recorded. For each plant species, 0.5 g of ground sample was added to 8 nylon bags which were then dried at 105°C for 24 h, after which dry sample plus bag weight was recorded. Duplicate nylon bags for each feed type were randomly allocated to one of the four digestion vessels, and therefore to one of the four inoculum treatments.

The microbial inoculum was prepared from rumen liquor collected from mature healthy sheep (RL, n = 4) using an oesophageal tube under mild vacuum or from fresh faeces collected from sheep, goats and camels (SF, GF and CF, n = 4/species respectively).

After collection, the rumen liquor and faecal samples were placed in an air-tight container and transported to the laboratory at 39°C. The faecal inoculum was prepared by homogenizing 40 g of faeces with 360 mL of warm, distilled water for 2 min under CO_2 and then filtering through double-layered cheese cloth directly into the pre-warmed digestion vessels. Each digestion vessel contained 400 mL of either RL, SF, GF or CF inoculum and 1.6 L of buffer solution. The buffer solution consisted of

Table 1. Chemical composition of plant species collected from rangelands of Southern Tunisia (% DM)*.

	Shrub species**										
	AC	AH	FT	IC	LG	NR	RV	SA	ST	SM	ZA
DM*** (g/kg)	356	302	401	311	380	232	204	140	209	147	135
OM (g/kg)	904	801	689	804	632	831	732	742	683	724	700
CP (g/kg)	94	125	73	41	105	96	105	126	115	165	75
NDF (g/kg)	552	467	377	716	372	441	238	397	381	414	394
ADF (g/kg)	340	348	195	471	198	384	157	188	212	255	223

*Adapted from Laudadio et al. (2009);
**AC, *Artemisia campestris* L.; AH, *Atriplex halimus* L.; FT, *Frankenia thymifolia* Desf.; IC, *Imperata cylindrica* L.; LG, *Limoniastrum guyonianum*; NR, *Nitraria retusa* (Forssk) Asch.; RV, *Reaumuria vermiculata* L.; SA, *Salicornia arabica* L.; ST, *Salsola tetragona* Del.; SM, *Suaeda mollis* (Desf.) Delile; ZA and *Zygophyllum album* L.;
***DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre;

Table 2. *In vitro* digestibility (%) of DM (IVDMD) and OM (IVOMD) of forages using different microbial inoculum sources[1].

	Shrub species*										
	AC	**AH**	**FT**	**IC**	**LG**	**NR**	**RV**	**SA**	**ST**	**SM**	**ZA**
IVDMD**											
RL	77.6	72.6	69.5	60.9	65.7[a]	77.8	60.1[a]	56.2[a]	32.4[a]	47.5	48.8
SF	76.5	73.0	67.3	58.4	62.5[a]	74.5	60.8[a]	58.9[a]	31.7[a]	44.5	47.4
GF	76.2	77.3	69.1	62.2	70.8[b]	75.1	68.6[b]	68.3[b]	48.1[b]	43.1	50.7
CF	76.6	75.6	68.4	62.5	73.6[b]	75.6	71.9[b]	70.0[b]	47.0[b]	45.6	51.5
CV[2]	1.18	2.32	1.01	1.89	3.11	2.79	3.44	3.03	4.25[b]	3.88	2.17
IVOMD***											
RL	63.0	68.0	64.9	66.3	61.1	61.2	65.5	61.6	37.8	52.9	50.2
SF	64.2	68.8	65.0	66.1	60.2	62.2	65.5	66.6	39.4	52.3	48.1
GF	62.5	68.7	65.5	69.6	67.2	63.0	68.0	64.7	54.4	59.5	57.0
CF	63.0	65.6	69.0	68.9	70.0	65.0	68.3	66.4	58.4	62.1	57.9
CV	1.44	1.11	1.94	1.51	2.12	1.23	2.02	1.16	4.07	3.32	1.97

[1] RL, rumen fluid from sheep; SF, faeces from sheep; GF, faeces from goat; CF, faeces from camel;
***AC, *Artemisia campestris* L.; AH, *Atriplex halimus* L.; FT, *Frankenia thymifolia* Desf.; IC, *Imperata cylindrica* L.; LG, *Limoniastrum guyonianum*; NR, *Nitraria retusa* (Forssk) Asch.; RV, *Reaumuria vermiculata* L.; SA, *Salicornia arabica* L.; ST, *Salsola tetragona* Del.; SM, *Suaeda mollis* (Desf.) Delile; ZA and *Zygophyllum album* L.;
**IVDMD, *in vitro* dry matter digestibility;
*** IVOMD, *in vitro* organic matter digestibility;
[2] Coefficient of variation (%);
Values in the same column with a different alphabetical superscript are significantly different ($P < 0.05$).

1.33 L buffer A (KH_2PO_4, 10.0 g/L; $MgSO_4.7H_2O$, 0.5 g/L; NaCl, 0.5 g/L; $CaCl_2.2H_2O$, 0.1 g/L; and urea, 0.5 g/L) and 266 mL of buffer B (Na_2CO_3, 15.0 g/L and $Na_2S.7H2O$, 1.0 g/L), mixed in each digestion vessel and the pH was adjusted to 6.8. Rumen liquor (400 mL) and SF, GF and CF (400 mL of each) were then added to the buffer solution in separate digestion vessels after which CO_2 was purged for 30 s and then sealed. The sealed digestion vessels were placed

into the pre-warmed Daisy[II] incubator. The incubator maintained a constant temperature of 40°C throughout the incubation while the digestion vessels were continuously agitated.

The digestion vessels were removed after 48 h and the filter bags were immediately rinsed for 30 min with cold water to stop microbial activity. Samples were analyzed for DM, OM, CP, NDF and ADF digestibility based on the methods of Goering and Van Soest (1970) and modified by ANKOM Technology.

The experimental design was completely randomized with 4 treatments (source of inoculum) × 11 feedstuffs. The effect of treatment was compared by analysis of variance procedures and the contrast of means by Tukey´s test, using SAS statistical software (SAS, 2000).

Table 3. *In vitro* digestibility (%) of CP (CP_d), NDF (NDF_d) and ADF (ADF_d) of forages using different microbial inoculum sources[1].

	Shrub species*										
	AC	**AH**	**FT**	**IC**	**LG**	**NR**	**RV**	**SA**	**ST**	**SM**	**ZA**
CP_d**											
RL	43.2	39.8	38.2	44.5	41.4	52.1	43.5	37.8	49.6	56.0	52.8
SF	42.5	41.1	38.9	45.2	41.0	49.5	43.9	39.1	48.2	55.7	52.9
GF	42.0	40.8	39.3	44.8	41.7	50.9	44.0	37.4	48.8	55.3	52.0
CF	42.9	40.5	39.0	43.1	41.7	51.2	44.1	39.7	47.4	55.1	51.9
CV[2]	1.22	1.12	1.01	1.15	1.32	1.28	1.31	1.46	1.49	1.31	1.17
NDF_d***											
RL	61.8	56.3	54.5	46.7	49.3	64.6	45.4	42.0 a	24.6 a	31.7	29.0
SF	60.5	59.0	53.0	50.0	52.0	65.2	45.8	40.7 a	24.7 a	34.6	30.4
GF	59.6	61.2	53.4	52.2	53.6	69.2	53.2	53.3 b	31.5 b	36.6	33.1
CF	59.0	62.2	55.5	49.6	52.3	65.2	50.1	52.8 b	31.0 b	35.5	31.1
CV	1.87	2.12	1.45	1.98	2.02	2.11	1.99	3.45	3.13	1.32	1.87
ADF_d****											
RL	42.0	38.8	35.9	29.6	31.9	45.9	28.6	26.2	10.3	14.0	11.4
SF	40.7	41.5	34.4	32.9	34.6	46.5	29.0	24.9	10.2	14.8	10.6
GF	39.8	43.7	34.8	35.1	36.2	49.3	31.4	27.5	12.2	15.8	12.2
CF	39.2	44.7	36.9	32.5	34,9	46.5	30.3	28.0	11.9	15.7	11.3
CV	1.21	1.02	2.06	1.98	1.01	1.32	1.12	1.44	1.89	1.77	1.69

[1] RL, rumen fluid from sheep; SF, faeces from sheep; GF, faeces from goat; CF, faeces from camel;

**AC, *Artemisia campestris* L.; AH, *Atriplex halimus* L.; FT, *Frankenia thymifolia* Desf.; IC, *Imperata cylindrica* L.; LG, *Limoniastrum guyonianum*; NR, *Nitraria retusa* (Forssk) Asch.; RV, *Reaumuria vermiculata* L.; SA, *Salicornia arabica* L.; ST, *Salsola tetragona* Del.; SM, *Suaeda mollis* (Desf.) Delile; ZA and *Zygophyllum album* L.;

**CP_d, in vitro crude protein digestibility;

*** NDF_d, *in vitro* neutral detergent fibre digestibility;

****ADF_d, *in vitro* acid detergent fibre digestibility;

[2] Coefficient of variation (%);

Values in the same column with a different alphabetical superscript are significantly different (P <0.05).

3. Results

Digestibility of DM, OM, CP, NDF and ADF of various feed samples using the Daisy^{II} incubation system are presented in Tables 2 and 3, respectively.

For the feeds studied in this experiment the range for IVDMD was 32-78%, for IVOMD was 38-70%, for CP_d was 38-56%, for NDF_d was 25-62% and for ADF_d was 10-49%. Estimates of DM, OM and NDF digestibility were higher with the faecal inoculum collected from the goat and camel for some plant species collected than for rumen liquor and faecal inoculum from sheep. Digestibility estimates from SF, GF, CF were more consistent across replicates compared with samples incubated with RL.

There was no difference in digestibility of all parameters for all plant species between RL and SF. Similarly GF and CF inoculum resulted in similar digestibility for all parameters for all plant species. However, the digestibility of DM of *I. cylindrical*, *L. guyonianum*, *R. vermiculata*, *S. arabica* and *S. tetragona* was greater when the samples were incubated in GF or CF compared with SF or RL. Further, the digestibility of OM of *L. guyonianum*, *R. vermiculata*, *S. mollis* and *Z. album* was greater when samples were incubated in GF or CF compared with SF or RL. In addition, the digestibility of NDF of *R. vermiculata*, *S. arabica* and *S. tetragona* were greater when incubated with GF or CF inoculum compared with SF or RL.

4. Discussion

A simple method for *in vitro* estimation of feed digestibility has been recently introduced (Mabjeesh et al., 2000), as an alternative to the traditional method of Tilley and Terry (1963), using the Daisy^{II} incubator (Ankom Tech Co., Fairport, NY, USA). This procedure permits a simultaneous incubation of an high number of samples (up to 96 samples per incubation) and does not adversely affect the precision and repeatability of the value obtained (Lattimer et al., 2007).

Estimates achieved using the Daisy^{II} incubator used in this trial can be interpreted as estimates of true digestibility of forages in the pasture (Laudadio et al., 2009). Since the residue resulting from the incubation *in vitro* for 48 h, is a mixture of undigested forage and rumen microorganisms and their subsequent step by neutral NDF microorganisms and remnants of cell content of forages, so that the cell wall and the time of the degradation of it, determines the value of the *in vitro* digestibility of the substrate. The *in vitro* digestibility with Daisy^{II} was different ($P < 0.05$) for the various forages (Table 2 and 3), maybe due to the order of increasing cell wall content (NDF) and ADF has been reported and more pronounced the higher fiber content. The similarity between the different repetitions for all the fodder in the estimation of nutrient digestibility in Daisy^{II} , reflects its accuracy, making it comparable to the digestibility values found with traditional method for many types of forages including grass forage, grass hay, legumes such

as alfalfa and silage (Holden, 1999) or concentrates and protein supplements (Mabjeesh et al., 2000). With the use of Daisy^{II} has reported a satisfactory accuracy of the digestibility of the technique *in vitro*, even greater than the method of Tilley and Terry (1963). Variation in our work, including all sources of variation was lower than the value found in the digestibility for Italian ryegrass and alfalfa (Wilman and Adesogan, 2000) using the same method. The repeatability obtained, defined as the variation among pitchers was between 0.22 and 3.06% depending on the type of forage.

The repeatability found was higher than reported in other work using the Daisy^{II} in the evaluation of 12 fodder species (Spanguero et al., 2003). Variations in the results of digestibility *in vitro* can be attributed to several factors, such as the processing of samples, the difference in chemical composition of food, the preparation of buffer solution, the handling of equipment and porosity of the filter bags. The Daisy^{II}, following the methodology of Goering and Van Soest (1970), is an effective system for estimating *in vitro* digestibility, producing data similar to traditional methodologies, enabling a faster processing without adversely affecting the precision of results (Holden, 1999).

In conclusion, the Daisy^{II} incubator provided a fast, accurate and simple method to determine the digestibility of a large number of feed samples compared with conventional methods. The *in vitro* digestibility measured with the Daisy^{II} system had a high repeatability making the procedure fast, easy and economical. The use of different faecal sources as inoculums for the Daisy^{II} incubator may permit this technique to be used in routine laboratory evaluations of the nutritive value of plant species, including those grazed by camels, reducing costs and risks regarding the use of fistulated animals and technical expertise required for rumen fluid collection via the oesophagus.

5. Acknowledgements

The present work was supported by the Italian and Tunisian cooperation funds. Thanks are due to the technicians of departments for their help during laboratory analysis.

References

Goering H.K., Van Soest P.J., 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379. ARS, USDA, Washington, DC.

Holden L.A., 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. J. Dairy Sci., 82, 1791-1794.

Jones R.J., Barnes P., 1996. In vitro digestibility assessment of tropical shrubs legumes using rumen fluid or faecal fluid as the inoculum source. Trop. Grassl., 30, 374-377.

Lattimer J.M., Cooper S.R., Freeman D.W., Lalman D.L., 2007. Effect of yeast culture on *in vitro* fermentation of a high-concentrate or high-fiber diet using equine fecal inoculum in a Daisy^{II} incubator. J. Anim. Sci., 85, 2484-2491.

Laudadio V., Tufarelli V., Dario M., Hammadi M., Seddik M.M., Lacalandra G.M., Dario C. 2009. A survey of chemical and nutritional characteristics of halophytes plants used by camels in Southern Tunisia. Trop. Anim. Health Prod., 41, 209-215.

Mabjeesh S.J., Cohen M., Arieli A., 2000. *In Vitro* Methods for Measuring the Dry Matter Digestibility of Ruminant Feedstuffs: Comparison of Methods and Inoculum Source. J. Dairy Sci., 83, 2289–2294.

Mauricio R.M., Owen E., Mould F.L., Givens I., Theodorou M.K., France J., Davies D.R., 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.

Minson D.J., 1990. Forage in Ruminant Nutrition. Academic Press Inc. San Diego, USA.

Murray J.M.D., Longland A., Dunnett C., 2008. Effect of yeast supplementation on the *in vitro* fermentation of high-temperature dried lucerne incubated with an equine faecal inoculum. Anim. Feed Sci. Technol., 146, 149–159.

Omed H.M., Lovett D.K., Axford R.F.E., 2000. Faeces as a source of microbial enzymes for estimating digestibility. In: Givens, D.I., Owen, E., Omed, H.M., Axford, R.F.E. (Eds.), Forage Evaluation in Ruminant Nutrition. CAB International, Wallingford, 135-154.

SAS, 2000. SAS/STAT Software: Changes and Enhancements through Release 8.1, SAS Institute Inc., Cary, NC.

Spanguero M., Boccalon S., Gracco L., Gruber L., 2003. NDF degradability of hays measured in situ and *in vitro*. Anim. Feed Sci. Technol., 104, 201-208.

Tilley J.M., Terry R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc., 18, 104-111.

Vàradyovà Z., Baran M., Zeleňák I., 2005. Comparison of two *in vitro* fermentation gas production methods using both rumen fluid and faecal inoculum from sheep. Anim. Feed Sci. Technol., 123-124, 81–94.

Wilman D., Adesogan A., 2000. A comparison of filter bag methods with conventional tube methods of determining the *in vitro* digestibility of forages. Anim. Feed Sci. Technol., 84, 33-47.