The effect of different grass-legume mixtures on nitrogen excretion of grazing sheep

Molle G., Giovanetti V., Cabiddu A., Cuccureddu M., Sitzia M., Decandia M.

in

Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). Nutritional and foraging ecology of sheep and goats

Zaragoza : CIHEAM / FAO / NAGREF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85

2009
pages 55-60

The effect of different grass-legume mixtures on nitrogen excretion of grazing sheep

G. Molle, V. Giovanetti, A. Cabiddu, M. Cuccureddu, M. Sitzia and M. Decandia

Istituto Zootecnico e Caseario per la Sardegna, AGRIS, 07040 Olmedo (Italy)

Abstract. A three-year study was carried out to assess the effect of different grass-legume mixtures on the N excretion of grazing Sardinian sheep in early-mid lactation (January-May). Replicate groups rotationally grazed plots of three grass-legume mixtures consisting of common grass (*Lolium rigidum*, Gaudin) and different legumes, namely: *Medicago polymorpha* (burr medic, treatment BM), *Trifolium subterraneum* (subclover, treatment SC) and *Hedysarum coronarium* (sulla, treatment SU). Herbage dry matter intake and faecal dry matter excretion were estimated by the n-alkane method in three sheep per replicate group on three or two occasions per year. The N content in herbage and faeces was measured in freeze-dried samples collected on the same occasions. N excretion in urine was estimated by difference, assuming no changes in body N while N utilisation efficiency (NUE) was calculated as the ratio between the yield of N in milk and N intake (NI). In years 1 and 3, corresponding to a higher proportion of legumes in sheep diet, SU groups showed higher N concentration and excretion in faeces and higher proportion of N in faeces as compared to the other groups ($P < 0.05$). In contrast, in the same years, BM resulted in a higher N excretion in urine ($P < 0.05$) and lower NUE ($P < 0.05$). Pooling all data ($n = 72$) NUE was negatively correlated with NI ($r = –0.804$, $P < 0.001$) and dietary N content ($r = –0.654$, $P < 0.001$). To conclude, the forage legume in the grass-legume mixture affected N excretion, its partitioning between urine and faeces and NUE.

Keywords. Legumes – Grazing – Sheep – N excretion – N efficiency.

Effets des différentes associations légumineuses-graminées sur l'excrétion azotée des brebis au pâturage

Résumé. *Une étude de trois ans a été menée pour évaluer les effets de différentes associations légumineuses-graminées sur l'excrétion d'azote, en début et milieu de lactation (janvier-mai), des brebis Sardes au pâturage. Pendant ces trois ans, chaque lot de brebis (répétition trois fois) a pâturé les trois types de parcelles composées de graminée commune (*Lolium rigidum*, Gaudin) et de différentes légumineuses, notamment* Medicago polymorpha *(luzerne polymorphe, lot BM),* Trifolium subterraneum *(trèfle souterrain, lot SC) et* Hedysarum coronarium *(sainfoin d'Espagne, lot SU). La matière sèche de l'herbe consommée et des fèces a été estimée par la méthode des n-alcanes sur trois brebis de chaque lot, deux à trois fois par an. Le taux d'azote de l'herbe et des fèces a été mesuré à partir d'échantillons lyophilisés. L'excrétion d'azote par l'urine a été estimée par différence, en ne supposant pas de changement du taux d'azote corporel. L'efficacité d'utilisation de l'azote (NUE) a été estimée par le rapport d'azote exporté dans le lait sur l'azote ingéré. Les rations les plus riches en légumineuses correspondent aux années 1 et 3 (effet année). Pendant ces deux années, les lots présents sur les parcelles SU ont présenté une concentration et une excrétion d'azote dans les fèces plus importante que les autres groupes ($P < 0,05$). Par contre les lots pâturant les parcelles BM, sur ces deux mêmes années, présentaient une importante excrétion d'azote par les urines ($P < 0,05$) et un faible NUE ($P < 0,05$). En regroupant toutes les données ($n = 72$) on peut conclure que le NUE est corrélé négativement avec l'azote ingéré NI ($r = –0,804$, $P < 0,001$) et le taux d'azote de l'herbe ($r = –0,654$, $P < 0,001$). Pour conclure, les pâturages associant graminées et légumineuses fourragères affectent l'excrétion d'azote, sa répartition entre urine et fèces et le NUE.*

Mots-clés. *Légumineuse – Pâturage – Brebis – Excrétion d'azote – Efficacité d'utilisation de l'azote.*

I – Introduction

There is an increasing awareness of the role played by agriculture activities inclusive of livestock production on environmental pollution. According to FAO (www.FAO.org) about 20% of the projected anthropogenic greenhouse effect is contributed by agriculture. A much higher proportion is estimated with reference to CH_4 (50%) and N_2O (70%) emissions. Furthermore livestock excreta contribute to runoff and leaching of N into water table with consequent eutrophication hazards. Although the livestock production systems based on pasture utilization are perceived as relatively benign with this respect, data on the release of N and other potential pollutants to the environment are too scanty to confirm this hypothesis. This is particularly the case for Mediterranean pastoral systems such as the dairy sheep grazing systems. The following study is part of a wide-framed multidisciplinary EU project (LEGGRAZE, www.univ-perp.fr/newsite/leggraze) aimed, among others, at evaluating the impact of legume-based grazing systems on N excretion and leaching. This report is focussed on assessing N excretion. The underlying hypothesis is that legume-based pastures that differ in agronomic features and chemical composition with particular reference to tannic phenols can result in different levels of N utilisation efficiency (NUE).

II – Materials and methods

The study was carried out at Bonassai research station (NW Sardinia, 41° N latitude, mean annual rainfall 582 mm). Three experiments were run in three grazing seasons falling, respectively in 2003 (January-May), 2004 (January-May) and 2005 (February-May). A randomized-block design was used with replicate plots or groups as experimental units.

Treatments consisted of three replicate plots (7500 m^2 each) of three grass-legume binary mixtures consisting of a common grass (*Lolium rigidum*, Gaudin, ecotype Nurra) and different legumes, namely: *Medicago polymorpha* (burr medic, cultivar Anglona, treatment BM), *Trifolium subterraneum* (subclover, cultivar Antas, treatment SC) and *Hedysarum coronarium* (sulla, cultivar Grimaldi, treatment SU). The pasture plots were established twice: in autumn 2002 and 2004 using the same seed rate for all legumes (35 kg/ha) but different seeding rates for the grass (14 and 10 kg/ha in year 2002 and 2004, respectively). On both occasions the plots were fertilised with 92 kg/ha of P_2O_5 before seeding. Prior to the beginning of the first experiment each plot was subdivided by metallic fences into two homogeneous sub-plots. A minimum of three "core" Sardinian mature ewes in early lactation (on average at 50-54 days in milk), were allocated to each treatment and replicate at the beginning of each experiment on the basis of live weight (mean ± SEM, 43.1 ± 0.6, 45.6 ± 0.8, and 44.1 ± 1.2 in experiment 1, 2 and 3, respectively), and milk yield (1904 ± 19, 2200 ± 20 and 1849 ± 53) as measured immediately prior to each experiment. These 9 groups rotationally grazed the corresponding sub-plots for 2 weeks, with the access to pasture unrestricted (approximately 22 h/d). Spare sheep, previously blocked for milk yield, were used to adjust stocking density to pasture growth using compressed sward height as the management criterion. In particular sward height was measured at the beginning and end of each grazing period with stocking density reduced if residual sward height was lower than 4-5 cm and increased if sward height exceeded 6-7 cm. Higher sward height targets (5-7 cm) were used during winter than during spring (4-6 cm). The sheep were machine milked twice daily at 7:30 and 15:30 CET.

At the beginning of each grazing period (see below) a minimum of three quadrats per plot (each of a minimum size of 0.5 m^2) were randomly cut to a residual height of 30 mm to measure botanical composition. Representative samples were taken and separated into sown legumes, grass and unsown species, which were then oven-dried to constant weight to determine relative plant species proportions on a dry-weight basis. Additional four 0.1 m^2 quadrats were cut to determine herbage mass. Detailed results on these measurements are reported by Sölter *et al*. (2007).

Three "core" sheep per replicate per treatment were used for intake and excretion measurements. Different animals were used each year. Intake was measured using the n-alkane marker method (Dove and Mayes, 1991) in three (2003 and 2004, January, March and end of April) or two periods (2005, March and end of April) per grazing season, aimed at representing early, mid and late vegetative phases of pasture. On each occasion the 3 "core" ewes in each group were dosed with a controlled release intra-ruminal bolus (CAPTEC, Auckland, New Zealand). The bolus released C_{32}

and C_{36} (n-dotriacontane and n-hexatriacontane, respectively) alkanes at a constant rate over approximately 21 days. Samples of faeces samples were collected per rectum from "core" animals twice daily after milking commencing on day 8 and ending on day 12 after dosing. Herbage samples, designed to be representative of material eaten by the animals were taken from the grazed horizon. This took place with a 36 hour lead time relative to collection of faeces to allow for passage through the gastro-intestinal tract. Grass and legumes were sampled separately.

Herbage (grass and legume) and faeces samples were freeze-dried, ground, bulked across collection days within each sheep, thoroughly mixed, and sub-samples analysed for concentration of C_{27}-C_{36} n-alkanes. Herbage dry matter intake (DMI, kg DM) and fecal excretion (FE, kg DM) were calculated as described by Dove and Mayes (1991). Sub-samples of bulked freeze-dried herbage and faeces were also analysed for nitrogen concentration (HN, g/kg DM and FN, g/kg DM, respectively), to allow for calculation of N intake [NI (g) = DMI x HN] and N excretion in feces [FNE (g) = FE x FN]. N excretion in urine (UNE) was estimated by difference (NI – yield of N in milk – FNE), assuming no changes in body protein. The proportion of N excretion in feces and urine was also computed. Sub-sample of herbage were also analysed for tannic phenols using the Folin-Ciocalteu method.

Individual milk yields were measured at morning and afternoon milking once a week during each intake measurement period and samples were taken to determine milk true protein (infrared method, Milkoscan 4000, Foss Electric, Hillerød, Denmark) and milk urea content determinations (enzymatic-colorimetric assay, Chem Spec 150, Bentley Instruments Inc. 4004 Peavey Road Chaska, MN 55318 USA). The N exported as milk was then calculated as the sum of protein and urea N. The N utilisation efficiency (NUE) was computed as percentage ratio between the yield of N in milk and N intake.

The data were firstly analysed within experiment by a general linear model which included the random effect of replicate (block) and the fixed effects of period within grazing season, treatment and their interaction. Treatment means were separated by Tukey test only if the treatment effect was significant at $P < 0.05$. Data were then pooled across experiments and treatments to assess the relationships between several independent variables and the N excretion variables using correlation models.

III – Results and discussion

The herbage mass (Fig. 1) was basically above limiting thresholds, the legume mass was very low in the second grazing experiment (year 2004), wherein only SU contributed a small proportion (4% DM) of legume. The average proportion of legume in the pastures ranged between 30 and 49% DM in experiment 1 and between 46 and 68% DM in experiment 3. Dietary tannic phenol concentration was consistently higher in the ewes grazing SU than in the counterparts (see Molle *et al*., 2009).

In both establishment years (Table 1) (experiment 1, 2003 and experiment 3, 2005), in which the legume proportion in the sward was relevant in all treatments, NI was higher in BM than the other treatments ($P < 0.01$, in experiment 1, $P = 0.06$ in experiment 3). In the same years, the estimated UNE was higher in the sheep grazing the burr medic-based mixture than in those grazing subclover or sulla-based pastures ($P < 0.01$, Table 1). In contrast, sheep grazing the sulla-containing pasture showed a higher FN (not shown) and FNE than their counterparts ($P < 0.01$). The proportion of N excreted in feces was also affected by the pasture mixture, being 55% (SU), 46% (SC) and 31% (BM) in experiment 1 and 51% (SU), 37% (SC) and 29% (BM) in experiment 3 ($P < 0.01$ for comparison between SU and the other two treatments in both years). The high proportion of N excreted as feces is in line with findings by Dentinho *et al*. (2006), who found that in sheep fed fresh sulla approximately 70% of excreted N was released in feces. In our study this result can be explained by a lower CP digestibility in sulla than the other legumes due to the presence of moderate amount of tannic phenols in this legume, as shown by Stienezen *et al*. (1996). As expected, BM in both establishment years showed a higher proportion of N excreted as urine ($P < 0.001$ between BM and the others in experiment 1 and only between BM and SU in experiment 3). In the establishment years (2003 and 2005) BM showed also higher total estimated N excretion and lower NUE, although for the latter variable SU in the first and SC in the third grazing seasons were undifferentiated from BM (Table 1). In lactating meat sheep continuously stocked at 3, 6 or 9 cm on grass-clover mixed sward, Parsons *et al*. (1991) found that average total N excretions ranged between 73 and 90 g/d with a proportion of N excreted in feces averaging 24%, which is slightly lower than that of BM group in this study.

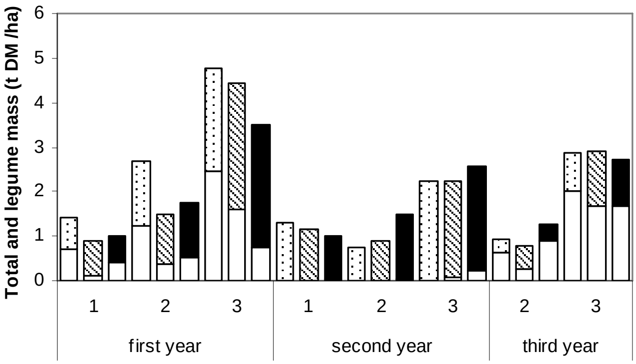

Fig. 1. Average total and legume mass (white bottom boxes) on offer in burr-medic (dotted bars), subclover (dashed bars) and sulla-based grass-legume binary mixtures (solid bars).

Table 1. Least square means for N intake, excretion and utilization efficiency (NUE) in milked sheep grazing grass-legume binary mixtures differing in the legume component (BM, burr medic, SC, subclover, SU, sulla)

	Treatment			P<		
	BM	SC	SU	Treat.	Period	T. x P.
Experiment 1 (2003)						
N intake (g)	80 a	56 b	62 b	***	**	NS
Urinary N excretion† (g)	46 a	23 b	24 b	**	NS	NS
Fecal N excretion (g)	19 a	18 a	24 b	***	NS	NS
Total N excretion (g)	65 a	42 b	48 b	***	*	NS
NUE (N in milk/N intake %)	19.28 a	25.54 b	23.49 ab	*	NS	NS
Experiment 2 (2004)						
N intake (g)	81	71	87	NS	***	NS
Urinary N excretion† (g)	48	41	52	NS	***	NS
Fecal N excretion (g)	18 ab	16 a	22 b	***	NS	*
Total N excretion (g)	66	57	74	NS	****	NS
NUE (N in milk/N intake %)	18.93 ab	21.70 a	15.62 b	*	***	*
Experiment 3 (2005)						
N intake (g)	83	67	76	0.06	NS	**
Urinary N excretion† (g)	49 a	33 b	30 b	***	NS	**
Fecal N excretion (g)	19 a	19 a	29 b	***	NS	0.06
Total N excretion (g)	69 a	52 b	59 ab	*	NS	**
NUE (N in milk/N intake %)	17.62 a	22.45 ab	23.36 b	*	NS	NS

†Estimated by difference: N intake – N yielded in milk – N excreted in feces.
a, b: means in rows with different letters differ at P < 0.05.

The treatment effect in year 2 (re-growth year for sulla and self-reseeding year for the other legumes) was smoothed by the low legume proportion in the pasture. Nevertheless the positive effect of sulla-based pasture on FN (not shown) and FNE was basically confirmed ($P < 0.05$) but this was not the case for the ranking of NUE which was worse in SU compared to SC with BM as intermediate. This was probably related to the trend to higher NI in SU groups ($P = 0.09$). In year 2 (2004), no effect of treatment was found on UNE, total N excretion, or the estimated partitioning of N excretion between feces and urine ($P > 0.1$).

Overall the period showed an effect on NI with a decreasing trend along with the season in the first two years and with an interaction ($P < 0.05$) with treatment in experiment 3, when SU showed a peak of NI in the last measurement period. A similar trend was evident for UNE (Fig. 2). FNE was affected by the interaction between treatment and period in the last two grazing seasons ($P < 0.05$ and $P < 0.06$, respectively) with only the SU groups showing an increase in the last measurement periods (Fig. 2). This is related to a late surge in herbage DM intake and hence NI as compared to the other treatment groups ($P < 0.01$ for the interaction).

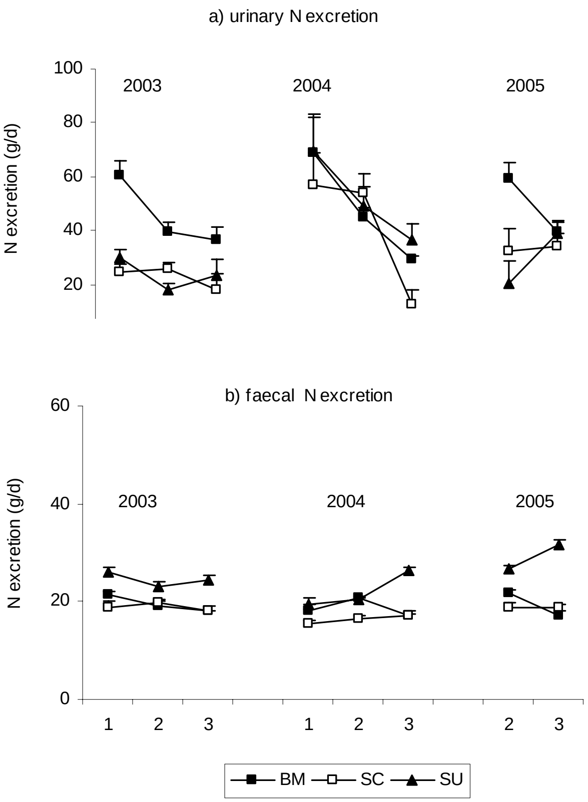

Fig. 2. Pattern of N excretion in milked sheep grazing grass-legume binary mixtures differing for the legume component (BM, burr medic, SC, subclover, SU, sulla). Least square means ± SE.

Pooling all the data of the study, inclusive of data on diet chemical composition and milk urea N (MUN) reported by Molle *et al*. (2009), an exploratory correlation analysis showed that UNE was strictly related with NI ($r = 0.973$, $P < 0.001$), N digestibility ($r = 0.846$, $P < 0.001$), dietary content of N ($r = 0.775$, $P < 0.001$), NFC ($r = –0.509$, $P < 0.001$) and WSC ($r = –0.419$, $P < 0.001$). A weaker relationship was also found with milk urea N ($r = 0.527$, $P < 0.001$). A much stronger correlation between UNE and MUN was found by Giovanetti *et al*. (2007) in lactating sheep fed pelleted concentrates differing in NFC content and source ($r = 0.969$, $P < 0.001$). A weak negative association between UNE and the dietary tannic phenol content was also detectable ($r = –0.308$, $P < 0.01$).

In contrast, tannic phenol content was the second best individual predictor of FNE ($r = 0.675$, $P < 0.001$) following FN ($r = 0.733$, $P < 0.001$). No correlations were found between FNE and either N content, N intake or milk urea N concentration. This is in apparent contrast with the results by Giovanetti *et al*. (2007) who found dietary NFC content and N digestibility were the best single predictors of FNE. However their results refer to virtually tannin-free pelleted diets. NUE, as expected, was strongly negatively correlated with dietary N intake ($r = –0.804$, $P < 0.001$), N digestibility ($r = –0.738$, $P < 0.001$) and dietary N concentration ($r = –0.654$, $P < 0.001$). A positive but weaker association was found instead with the NFC content ($r = 0.429$, $P < 0.001$).

IV – Conclusions

Grazing a sulla-based pasture with a moderate level of tannic phenols increased FN and FNE. In contrast grazing a burr medic-based pasture resulted in higher NI and UNE and overall in lower NUE, at least in the years wherein legume proportion in the pasture was relevant. Tannic phenols confirmedly play a role in modulating fecal N excretion. To conclude, this study suggests that pastures based on forage legumes containing tannic phenols (sulla) or with a relatively low protein content (subclover) are more benign to the environment with reference to N losses through *excreta* than pastures based on tannin-free, high protein legumes such as burr medic.

Acknowledgements

This project has been funded by the European Union (LEGGRAZE – QL K5-CT-2001-02328). Mr. A. Pintore and Mr. S. Picconi and all the staff of the laboratory of feedstuffs analysis are acknowledged for their technical assistance.

References

Dentinho M.T.P., Bessa R.J.B., Belo C.A. and Ribeiro J.M.R., 2006. Effect of preservation method on the nutritive value of sulla (*Hedysarum coronarium*). In: *Grassland Science in Europe*, 11. p. 252-254.

Dove H. and Mayes R.W., 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A review. In: *Aust. J. Agric. Res.*, 42. p. 913-952.

Giovanetti V., Decandia M., Boe F., Zerbini E., Cannas A. and Molle G., 2007. Nitrogen excretion and utilization efficiency in dairy sheep fed diets with different dietary energy contents. In: *J. Anim. Sci.*, 85, Suppl. 1. p. 617.

Molle G., Giovanetti V., Cabiddu A., Cuccureddu M., Scanu G. and Decandia M., 2009. Milk urea as nutritional indicator in sheep grazing legume-based pastures. *Options Méditerranéennes, Series A*. This volume.

Parsons A.J., Orr R.J., Penning P.D. and Lockyer D.R., 1991. Uptake, cycling and fate of nitrogen in grass-clover swards continuously grazed by sheep. In: *J. Agric. Sci. Camb.*, 116. p. 47-61.

Sölter U., Hopkins A., Sitzia M., Goby J.P. and Greef J.M., 2007. Seasonal changes in herbage mass and nutritive value of a range of grazed legume swards under Mediterranean and cool temperate conditions. In: *Grass and Forage Sci.*, Vol. 62, Issue 3. p. 372-388.

Stienezen M., Waghorn G.C. and Douglas G.B., 1996. Digestibilty and effects of condensed tannins on digestion of sulla (*Hedysarum coronarium*) when fed to sheep. In: *New Zeal. J. Agric. Res.*, 39. p. 215-222.