

# Comparative studies on microbial protein synthesis in the rumen of goats and sheep

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## ABSTRACT

The ruminal microbial protein synthesis (MPS) was estimated in six adult Granadina goats and six Segureña sheep by measuring the urinary allantoin excretion and using the purine bases/N (PB/N) content ratios, determined in liquid associated bacteria (LAB) from continuous fermentors. Both, animals and fermentors were fed four different diets which included olive by-products: two-stage olive cake and olive leaves. Goats showed higher ( $P<0.001$ ) microbial protein synthesis and efficiencies values for some diets than sheep. The PB/N content was affected ( $P<0.001$ ) by the quality of diet supplied to the fermentors and its use resulted in an overestimation of MPS.

KEY WORDS: microbial protein, goats, sheep, purine derivatives

## INTRODUCTION

An intensive research labour over the last 30 years has been carried out to estimate microbial protein synthesis (MPS) in ruminants. The information has been generally obtained by using balanced rations, formulated with conventional feedstuffs, but little or any information is available concerning non conventional feeds or industrial by-products. Almost null comparative interspecies (goats vs sheep) studies are available, concerning MPS. Several endogenous and exogenous markers to label microbial material have been used, being the urinary excretion of purine derivatives (PD) the most useful approach for estimating MPS in ruminants (Balcells et al., 1991), in the last years. Recently, a response model using the urinary excretion of purine derivatives (PD) has been developed in goats (Belenguer et al., 2002) which allows us to estimate the MPS also in this specie.

The objective of this work was to compare the microbial protein supply to the duodenum and its efficiency of synthesis in sheep and goats, fed different

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quality diets which included olive by-products, by using the PD response models developed for each animal species.

## MATERIAL AND METHODS

Six non cannulated Granadina goats ( $46 \pm 3.1$  kg BW) and six non cannulated Segurena wethers ( $72 \pm 5.1$  kg BW) were randomly fed four experimental diets consisting of lucerne hay -AH-; lucerne hay (60%) plus pellets (40%), formulated with two stage olive cake -TSDOC- and barley grain (1:2) -AHCO-; olive leaves -OL-, and OL (66%) plus barley grain (23%) and faba bean (10%) -OLSUP-. Diets included a mineral-vitamin mixture (7%) to ensure animals requirements and were offered once daily at 09.00. After 20 d of adaptation to the experimental diet, the animals were disposed in individual metabolism crates. Samples of feeds and daily refusals were taken for each animal during 7 d, thawed and mixed before analysis. Urine was collected (under 10%  $H_2SO_4$ ) daily, before feeding, during 5 d. After collection 100 ml sub-sample was stored at  $-20^\circ C$  for allantoin concentrations analyses. A parallel *in vitro* experiment, using eight continuous fermentors, was carried out. Fermentors were fed the same experimental diets supplied to the animals and inoculated with rumen liquor from goats (4 fermentors) and wethers (4 fermentors). At the end of the assay, liquid associated bacteria (LAB) isolation from fermentors content was carried out (Molina Alcaide et al., 1996). Bacterial pellets were freeze-dried and analysed for purine bases (Balcells et al., 1992) and N.

Samples of feeds, refusals and effluents were mill ground (1 mm) and analysed for DM, OM, CF and N, according to the AOAC (1984) methods. Gross energy (GE) was determined in an adiabatic calorimeter; NDF, ADF and ADL analyses were performed by the procedure of van Soest et al. (1991). Duodenal flow of purine bases (PB) ( $x$ ,  $\mu mol/kg LW^{0.75}$ ) was calculated from urinary PD excretion ( $y$ ,  $\mu mol/kg LW^{0.75}$ ), by following the models proposed by Balcells et al. (1991) and Belenguer et al. (2002) for sheep and goats, respectively. The  $x$  values, calculated for each animal, were transformed to microbial N supply (g/day) as:  $x / (0.83 \times z)$  where 0.83 is the true digestibility of duodenal PB and  $z$  the PB:N average content ratio (mmol:g) found in LAB, extracted from the fermentors fed the corresponding diet. Data were analysed by the GLM procedure of SAS (1989).

## RESULTS AND DISCUSSION

Diets based on lucerne hay (AH and AHCO), compared to those based on olive leaves (OL and OLSUP), showed higher N contents (30.0 and 25.4 vs 11.9 and 14.1, g/kg DM, respectively) and lower lignin (54.5 and 58.5 vs 167 and 127, g/kg DM, respectively), CF (17 and 24 vs 80.3 and 63.0, g/kg DM, respectively) and acid detergent insoluble N (62.0 and 107 vs 278 and 216, g/kg total N) contents. Table 1

shows the PB/N content ratios in LAB, harvested from the fermenters and the estimated microbial protein N supply to the duodenum and the efficiencies of synthesis.

The microbial N supply to the duodenum showed higher values than those found by others (Ben Salem et al., 2000) with sheep, using similar quality diets and the same microbial marker. In fact, the estimated efficiencies of synthesis were also higher than those proposed by either the ARC (1980) -32 g microbial N/kg DOMR-, AFRC (1992) -9 g microbial protein/MJ MFE- and Madsen et al. (1995) -20 g of amino acidic microbial N/kg CHOD-. This overestimation could be explained by either the use of a PB/N value obtained from fermenters, rather than from an inoculum collected directly from the rumen and the use of a bacterial sample as rumen protozoa presents 50% lower PB/N ratio values than bacteria. The PB/N ratio also presents differences ( $P<0.001$ ) depending on the diet supplied. However, they were not different ( $P<0.001$ ) between fermenters inoculated with rumen liquor from goats and sheep.

Table 1. Purine bases/nitrogen (PB/N) ratios in LAB, microbial protein supply to the duodenum and its efficiency of synthesis in goats and wethers fed the experimental diets<sup>1</sup>

Diet	Animal species	Microbial N				
		PB/N mmol/g	LW <sup>0.75</sup> g/kg	DOMR g/Kg	MFE g MP/MJ	g N aa LAB/kg CHOD
AH	Goats	0.70	1.10	41.5	14.8	26.1
	Sheep	0.80	0.86	39.5	13.8	23.6
AHCO	Goats	0.47	1.53 <sup>a</sup>	73.1 <sup>a</sup>	22.0 <sup>a</sup>	43.0 <sup>a</sup>
	Sheep	0.51	0.89 <sup>b</sup>	51.9 <sup>b</sup>	16.1 <sup>b</sup>	29.6 <sup>b</sup>
OL	Goats	0.50	0.52	44.7 <sup>a</sup>	33.1	24.4 <sup>a</sup>
	Sheep	0.53	0.49	37.1 <sup>b</sup>	29.9	19.6 <sup>b</sup>
OLSUP	Goats	0.58	1.01	42.5	19.0	23.2
	Sheep	0.61	0.95	42.1	21.4	22.9
Significance level <sup>2</sup>	AS	NS	**	**	NS	*
	D	***	***	**	***	***
	AS x D	NS	NS	NS	*	*

<sup>1</sup> AH: lucerne hay; AHCO: lucerne hay plus concentrate (barley grain and two-stage olive cake);

OL: olive leaves; OLSUP: olive leaves, barley grain and faba bean

<sup>2</sup> AS: animal species effect; D: diet effect

<sup>a,b</sup> in a row, for each experimental diet, means without a common superscript letter differ ( $P<0.05$ )

Goats showed higher ( $P<0.001$ ) microbial protein supply to the duodenum per kg/LW<sup>0.75</sup> than wethers when they were fed AHCO diet. Goats also showed higher efficiencies of synthesis than wethers when fed AHCO and OL diets, indicating a most efficient microbial activity in the rumen of this animal species.

When MSP was correlated with the parameters proposed by the different Protein Evaluation Systems of Feedstuffs for Ruminants, as an index of energy availability for rumen microorganisms, the highest relationships were obtained with CHOD ( $r = 0.85$ ) and MFE ( $r = 0.83$ ) intakes, compared to that related with DOMR ( $r = 0.78$ ). It might be due to the dietary fat correction that both approaches make (AFRC, 1992; Madsen et al., 1995) as OL and OLSUP diets presented relatively high fat contents (80.0 and 60.3 g/kg DM, respectively).

## CONCLUSIONS

Sheep and goats showed differences in microbial protein synthesis and efficiencies depending on diet quality. That could indicate the need of knowing this parameter separately for each species for accurate diets formulation. PB/N content ratios in the rumen bacteria change with diets quality variation. The use of values from LAB isolated in continuous fermentors, gives a clear overestimation of the microbial protein synthesis.

## REFERENCES

- AFRC, 1992. Technical Committee on Responses to Nutrients: Report No. 9. Nutr. Abst. Rev., Ser. B 62, 787-835
- AOAC, 1989. Official Methods of Analysis, Association of Official Agricultural Chemists. 14<sup>th</sup> Edition. Washington, DC
- ARC, 1980. The Nutrients Requirements of Ruminant Livestock. Royal, CAB
- Balcells J., Guada J.A., Castrillo C., Gasa J., 1991. Urinary excretion of allantoin and allantoin precursor by sheep alter different rates of purine infusion into the duodenum. *J. Agr. Sci.* 116, 309-317
- Balcells J., Guada J.A., Peiró J.M., Parker D.S., 1992. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography. *J. Chromatogr.* 575, 153-157
- Belenguer A., Yáñez D., Balcells J., Ozdemir Baber N.H., González Ronquillo M., 2002. Urinary excretion of purine derivatives and prediction of rumen microbial outflow in gotas. *Livest. Prod. Sci.* 77, 127-135
- Ben Salem H., Nefzaoui A., Ben Salem L., Tisserand J.L., 2000. Deactivation of condensed tannins in *Acacia cyanophylla* Lindl. foliage by PEG in feed blocks. Effect on feed intake, nitrogen balance, microbial synthesis and growth by sheep. *Livest. Prod. Sci.* 64, 51-60
- Madsen J., Hvelplund T., Weisbjerg M.R., Bertilsson J., Olsson I., Spörndly R., Harstad O.M., Volden H., Tuori M., Varvikko T., Huhtanen P., Olafsson B.L., 1995. The AAT/PBV protein evaluation system for ruminants. A revision. *Norwegian J. Agr. Sci., Suppl.* 19, 37
- Molina Alcaide E., Weisbjerg M.R., Hvelplund T., 1996. Degradation characteristics of shrubs and the effect of supplementation with urea or protein on microbial production using a continuous culture system. *J. Anim. Physiol. Anim. Nutr.* 75, 121-132
- SAS. 1989. SAS/STAT® User's guide Int. (Version 6,4<sup>th</sup> Edition)
- Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583-3597