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Effect of PEG supply on rumen degradability of a concentrate in a continuous culture system

A. Moumen, D.R. Yáñez Ruiz, A.I. Martín García and E. Molina Alcaide
Department of Animal Nutrition, Estación Experimental del Zaidín (CSIC)
Camino del Jueves s/n, 18100 Armilla, Granada, Spain

SUMMARY – The *in sacco* procedure was used to determine dry matter (DM) and crude protein (CP) degradability of a concentrate at different incubation times in a continuous culture system, inoculated with rumen liquor from sheep or goats. The concentrate degradability was determined in the presence and absence of a tannin-binding agent: polyethylene glycol (PEG) 4000 MW. The fermenters were fed alfalfa hay (AH) and a concentrate (CO) (60:40). The concentrate was composed of two stage olive cake, barley grain (1:3) and a mineral-vitamin mixture. Total condensed tannin (TCT) content of alfalfa hay, barley grain, two stage olive cake and concentrate was 4.90, 10.7, 37.3 and 7.31 g/kg DM, respectively. In the absence of PEG, rumen liquor from sheep and goats did not promote significant differences ($P>0.05$) for any parameter of concentrate rumen degradability. PEG increased potentially degradable fraction (*b*) ($P<0.05$ and $P<0.01$ for DM and CP, respectively), but decreased ($P<0.001$) the rapidly degradable fraction (*a*). However, DM and CP effective degradability (ED) of the concentrate was not increased by PEG addition. This could be due to the adaptation of ruminal micro-organisms against tannins or to the low tannins content in the concentrate. The lack of PEG supply effect on ED could be also due to an excess of nitrogen in the fermenters in relation to the micro-organism requirements.

Keywords: Continuous culture, degradability, PEG, concentrate, two stage olive cake.

RESUME – *"Effet de la supplémentation avec PEG sur la dégradabilité ruminale d'un concentré dans un système de culture continu". La dégradabilité de la matière sèche (DM) et de l'azote d'un concentré a été mesurée* in sacco *aux différents temps d'incubation dans un système de culture continu inoculé avec du jus de rumen de moutons ou de chèvres. La dégradabilité a été déterminée en présence ou en absence d'un agent bloquant des tannins : le polyéthylène glycol (PEG) 4000 MW. Les fermenteurs ont été alimentés avec du foin de luzerne et du concentré (60:40). Ce dernier était composé par du grignon d'olive de deux phases, des grains d'orge (1:3), et d'un mélange de minéraux et de vitamines. Les tannins condensés totaux (TCT) du foin de luzerne, des grains d'orge, du grignon d'olive de deux phases et du concentré ont été de 4,90, 10,7, 37,3 et 7,31 g/kg DM, respectivement. En absence de PEG, le jus du rumen provenant de moutons ou de chèvres n'influence aucun des paramètres de la dégradabilité du concentré. L'addition de PEG augmentait significativement la fraction potentiellement dégradable* (b) *(P<0,05 et P<0,01 pour la matière sèche et l'azote, respectivement), et elle diminuait significativement (P<0,001) la fraction rapidement dégradable* (a). *Cependant, la dégradabilité effective (ED) de la matière sèche et des matières azotées totales n'a pas augmenté avec l'addition de PEG. Cela peut être dû à la tolérance des micro-organismes ruminaux aux tannins ou à la faible concentration des tannins dans le concentré. L'absence d'effet de l'addition de PEG sur l'ED peut être aussi due à l'excès d'azote dans les fermenteurs par rapport aux besoins des micro-organismes.*

Mots-clés : *Fermenteurs à flux continu, dégradabilité, PEG, concentré, grignons d'olive de deux phases.*

Introduction

Olive culture and its derived industry have a great importance, from an economic and social point of view, in Mediterranean countries. A new procedure for olive oil extraction was established in Spain in 1995. The new technology of olive oil extraction generates a new by-product called two-stage olive cake (TSDOC). The re-cycling of this by-product is of great interest as its storage causes serious environmental and economic problems. However, the presence of phenolic compounds like tannins may limit the nutrient availability of the by-product (Aharoni *et al.*, 1998; Martín García, 2001) and also its rumen degradability (Salawu *et al.*, 1999; Martín García, 2001). Animals use several mechanisms to counteract the negative effect of ingested tannins, and ruminal microbes resistant to high levels of tannins, either singly or in a consortium, may constitute a unique part of this response (Miller *et al.*, 1995). Although the ability of the rumen microbial population to withstand high concentrations of plant

phenolics has received minor consideration (Lowry *et al*., 1996), recent studies indicated the existence of bacteria able to grow on forages with high tannin content (Nelson *et al*., 1995; Molina *et al*., 1999; McSweeny *et al*., 2001).

The objective of the present study was to evaluate the effect of PEG as tannin-binding agent on DM and CP degradability of a concentrate including TSDOC in continuous culture fermenters inoculated with rumen liquor from sheep or goats.

Materials and methods

A continuous culture system following the Miettinen and Setälä (1989) design has been used. Ruminal inoculum was obtained 2 h after feeding from three ruminally fistulated goats or sheep fed on a diet based on alfalfa hay and concentrate 60:40 in absence (AHCO) or presence (AHCOP) of PEG (2 g/1 g TCT). The concentrate (CO) was made with three parts of barley grain (BG), one part of two stage dried olive cake (TSDOC) and a mineral-vitamins mixture (20 g/kg of the concentrate). The TSDOC is a solid by-product obtained from the desiccation, extraction and partial pitting of crude olive cake produced after extracting oil by olives centrifugation in two phases. After being strained through two layers of cheesecloth, 700 ml of rumen fluid were added to the fermenters. Four fermenters were inoculated with rumen liquor from sheep and four with rumen liquor from goats. Fermenters were fed the same diet fed to donor animals. Artificial saliva (McDougall, 1948) was continuously infused into each fermenter at a dilution rate of 40 ml/h. Dry matter (DM) and crude protein (CP) degradability of the concentrate was estimated using nylon bags for samples incubation. After a 11 day adaptation period, bags (4 x 5 cm; 46 µm of pore size), containing 1.4 g of concentrate, were incubated into the fermenters for 0, 4, 8, 16, 24, 48 and 72 hours. The degradation parameters were determined using the Ørskov and McDonald (1979) exponential model. The effective degradability (ED) was calculated as $ED = a + [b * c/(c + k)]$, were *a* is the soluble fraction, *b* the potentially degradable insoluble fraction and *c* the degradation rate of *b.* The artificial saliva flow is considered as passage rate (*k)* value.

The DM, organic matter (OM), CP, crude fat (CF), gross energy (GE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and nitrogen attached to the acid-detergent fibre (N-ADF) contents were analysed according to the methodology described in a previous work (Martín García *et al*., 2003). Free, protein-bound and fibre-bound condensed tannins (CT) were determined using the Terrill *et al*. (1992) method modified by Pérez-Maldonado and Norton (1996).

The effects of the animal species and type of diets were analysed by the GLM procedure using a two-way ANOVA analysis. Treatment means were separated by *t-test* for multiple comparisons. The differences between groups were obtained using the Bonferroni test.

Results and discussion

Chemical composition of TSDOC, BG, CO and AH is shown in Table 1. The highest total CT was found in TSDOC (37.3 g/kg DM), while the lowest was found in AH (4.90 g/kg DM).

The DM and CP degradation parameters of the concentrate are shown in Table 2. The rumen inoculum origin had not effect ($P>0.05$) on any parameters of the rumen degradability. PEG addition decreased ($P<0.001$) the rapidly degradable fraction (*a*), and increased significantly the potentially degradable fraction (*b*) ($P<0.05$ and $P<0.01$ for DM and CP, respectively). This effect was observed also by Ben Salem *et al.* (1999) in sheep consuming a rich tannin shrub (*Acacia cyanophylla*). The degradation rate (*c*) was also increased by PEG addition although not significantly ($P>0.05$). Aharoni et al. (1998) indicated that the effect of tannins on the CP degradability was due to a lower degradation rate. However, the concentrate ED of DM did not increased and the one of CP decreased significantly by PEG addition. This effect could be due to the microorganisms adaptation against tannins, to the low tannins concentration in the concentrate or to an excess of nitrogen in the fermenters in relationship to the micro-organisms requirements.

Table 1. Chemical composition of two-stage dried olive cake (TSDOC), barley grain (BG), concentrate (CO) and alfalfa hay (AH)

	TSDOC	BG	CO	AH
Dry matter (g/100 g fresh matter)	87.6	91.2	90.0	91.2
g/100 g dry matter				
Organic matter	88.9	97.5	94.1	88.2
Crude protein	9.88	10.3	11.8	18.3
N-ADF (g/100g TN)	47.0	5.20	14.8	7.68
Crude fat	0.25	1.99	1.75	1.76
Neutral detergent fibre	63.2	26.3	37.8	39.0
Acid detergent fibre	43.0	6.74	15.8	24.6
Acid detergent lignin	23.6	1.32	5.63	4.78
Gross energy (MJ/kg DM)	18.9	21.1	18.6	17.8
Condensed tannins (g/kg dry matter)				
Free	4.64	8.53	3.54	1.09
Protein-bound	19.2	2.01	2.20	2.81
Fibre-bound	13.5	0.18	1.55	1.01
Total	37.3	10.7	7.31	4.90

Table 2. Dry matter (DM) and crude protein (CP) degradation parameters of the concentrate in continuous culture fermenters, inoculated with rumen liquor from sheep or goats, and fed alfalfa hay and concentrate without (AHCO) or with PEG supply (AHCOP)

		AHCO		AHCOP		Animal species			AHCO			A x P	S.E.M
		Sheep	Goats	Sheep	Goats	Sheep	Goats	LS	PEG -	PEG +	LS		
a (%)	DM	62.2	62.7	49.2	49.2	55.7	55.9	NS	62.4	49.1	***	NS	0.199
	CP	65.0	65.4	44.7	44.1	54.9	54.8	NS	65.2	44.4	***	NS	0.338
b (%)	DM	14.4	18.6	22.6	23.0	18.5	20.8	NS	16.5	22.8	*	NS	1.061
	CP	19.9	21.7	48.1	29.5	34.0	25.6	NS	20.8	38.8	**	*	2.437
c (h^{-1})	DM	0.067	0.115	0.175	0.122	0.121	0.119	NS	0.091	0.149	NS	NS	0.016
	CP	0.054	0.045	0.065	0.079	0.059	0.062	NS	0.050	0.072	NS	NS	0.006
PD (%)	DM	76.6	81.3	71.8	72.2	74.1	76.8	NS	78.9	72.0	*	NS	1.141
	CP	85.0	87.1	92.7	73.5	88.9	80.3	NS	86.1	83.2	NS	*	2.26
ED (%)	DM	62.1	74.9	67.0	66.3	64.6	70.6	NS	68.5	66.7	NS	*	1.532
	CP	76.6	78.5	66.2	63.0	70.8	71.4	NS	77.6	64.6	***	NS	0.925

PEG: without PEG; PEG +: with PEG; *a*: soluble fraction; *b*: potentially degradable insoluble fraction; *c*: degradation rate of fraction *b*; PD: potential degradability; ED: effective degradability, calculated as $a+[b * c/(c+k)]$, where $k = 0.042$ h^{-1} (= artificial saliva outflow); LS: level of significance; A x P: animal species x PEG interaction; NS: non significant (P>0.05); *: P<0.05; **: P<0.01; ***: P<0.001; SEM: standard error of the mean.

Conclusions

The results of the present study suggest that the effects of PEG could depend on the source of rumen inoculum, as some degradation parameters were improved by PEG addition only when fermenters were inoculated with rumen content from sheep.

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