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Original article

Effect of polyethylene glycol, urea and sunflower meal supply on two-stage olive cake fermentation

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Abstract – A continuous culture system, inoculated with rumen liquor from goats or sheep, was used to study the ruminal fermentation characteristics of the two-stage dried olive cake (TSDOC). The effects of adding polyethylene glycol (PEG; 0, 2 or 20 g per 100 g TSDOC) and/or supplementing with urea (U) or sunflower meal (SM) (1.5 g N per 100 g OM) were also studied. TSDOC fermentation promoted low volatile fatty acid (VFA) production (20.5 mmol per day), predominantly acetic acid, and low efficiency of VFA production (1.88 mol per kg digestible carbohydrates, DCHO). Both values increased ($P < 0.001$) with PEG and N addition, while no important differences ascribed to the origin of rumen inoculum were observed. Total and amino acid nitrogen output was low and increased ($P < 0.001$) with N addition without being affected by PEG treatment. Bacterial nitrogen production was calculated from the difference between non ammonia (NAN) and acid-detergent-insoluble nitrogen (ADIN) outputs. No important differences, deriving from the inoculum origin, were observed for bacterial N production rate and efficiency (g N per kg DCHO). Increased ($P < 0.001$) VFA, DCHO and bacterial N production and efficiency of VFA production were observed with N addition. The response to protein (i.e. sunflower meal) supply was more pronounced than to urea. Treatment of TSDOC with PEG increased VFA production ($P < 0.001$) (from 21.8 to 27.8 mmol per day) and efficiency of microbial N synthesis ($P < 0.01$) (from 17.7 to 27.1 g bacterial N per kg DCHO) but it decreased ($P < 0.001$) the DCHO production.

two-stage olive cake / continuous fermenters / PEG / nitrogen supplementation / tannins / sheep / goats

Résumé – Effet de l'addition de polyéthylène glycol supplémenté avec de l'urée ou du tourteau de tournesol sur la fermentation des grignons d'olive issus d'une centrifugation à deux phases. Un système in vitro en fermenteur continu, inoculé avec du jus de rumen de chèvre ou de mouton, a été utilisé pour étudier les caractéristiques de la fermentation ruminale des grignons d'olive issus d'une centrifugation à deux phases (TSDOC). L'effet de l'addition de polyéthylène glycol (PEG : 0, 2 ou 20 g par100 g TSDOC) supplémenté ou non soit avec de l'urée (U) soit avec du tourteau de tournesol (SM) (1,5 g N par 100 g MO) a également été estimé. La fermentation des grignons d'olive

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a diminué la production des acides gras volatils (20,5 mmol par jour), principalement l'acide acétique, ainsi que l'efficience de leur production (1,88 mol par kg glucides digestibles, DCHO). Ces deux valeurs ont augmenté ($P < 0,001$) avec l'addition du PEG et l'apport azoté ; en revanche, l'origine du jus de rumen n'a eu que très peu d'effet. Les productions de matières azotées totales et d'acides aminés ont été faibles, mais elles ont augmenté ($P < 0,001$) avec l'addition d'azote, tout en n'étant pas affectées par le traitement avec le PEG. La quantité de matières azotées d'origine bactérienne a été calculée à partir de la différence entre la production d'azote non ammoniacal et la production d'azote insoluble dans le détergent acide (ADIN). La vitesse de production et l'efficacité de synthèse (g N par kg DCHO) d'azote microbien n'ont pas été affectées par l'origine du jus de rumen, alors que la supplémentation azotée a augmenté ($P < 0,001$) les quantités d'acides gras volatils, des glucides fermentés, d'azote microbien, ainsi que le rendement de production des acides gras volatils. La réponse obtenue avec le tourteau de tournesol a été plus prononcée que celle obtenue avec l'urée. Le traitement des grignons d'olive avec le PEG a augmenté ($P < 0,001$) la quantité des acides gras volatils (de 21,8 à 27,8 mmol par jour) et la production d'azote microbien ($P < 0,01$) (de 17,7 à 27,1 g d'azote microbien par kg DCHO), mais il a diminué ($P < 0,001$) la quantité de glucides fermentés.

grignon d'olive / centrifugation à deux phases / système de culture continu / PEG / supplémentation azotée / tannins / mouton / chèvre

1. INTRODUCTION

Olive crop and derived industries are economically and socially very important in Mediterranean countries, especially in Spain, which is the first world producer. A new procedure for olive oil extraction called "two-stage" centrifugation has been used in Spain since 1995. This procedure generates a by-product called two-stage olive cake that includes olive skins, pulp and stones, whose total production is close to 3 million metric tons per year. It also includes vegetable waters, which were obtained separately in a previous extraction process (three-stage technology), being rich in polyphenols [15, 30]. This new by-product could be an interesting source of nutrients for livestock [23] since the scarcity of conventional feedstuffs in Mediterranean countries represents a limitation for animal production development. The re-utilisation of the two-stage olive cake may, in addition, have beneficial effects on the environment, since their storage and removal represent serious environmental problems [44].

Information on the nutritive value of the new two-stage olive cake is scarce [23], but in general is similar to that of the olive cake obtained by the three-stage technology [27]. To some degree, the low nutritive value of this olive by-product could be explained by the presence of phenolics such as tannins, which may limit nutrient availability [1, 23, 34, 39] due to their capacity to bind proteins and carbohydrates [32, 40]. The use of compounds such as polyethylene glycol (PEG), which seems to dissociate the tannin-protein complexes, could help to clarify the presence and effects of tannins in two-stage olive cake on ruminant digestion. Since it has been reported that sheep and goats have a different capacity for adapting to diets containing tannins [38], a comparative study of the inoculum source is relevant to state the value of the new two-stage olive cake for the two ruminant species.

In addition, the nutritive value of lignocellulosic feedstuffs may often be improved by adding supplementary nitrogen. The suitability of different forms of nitrogen to be used with lignocellulosic materials in promoting improved digestion is of great interest. Nevertheless, it is still lacking in clarity, especially related to the aptness of using protein or non-protein nitrogen [9, 26, 31].

The aim of the present work was to evaluate the qualitative and quantitative characteristics of the ruminal fermentation promoted by the new two-stage olive cake using a continuous culture system, inoculated with rumen liquor from goats or sheep. The effects

Table I. Ingredient composition of the experimental diets (g per kg fresh weight) based on two stage dried olive cake (TSDOC).

	Ingredients			
Diets	TSDOC	Polyethylene glycol	Urea	Sunflower meal
A	1000			
B	980	20		
C	833	167		
D	973		27	
E	779			221
F	955	19	27	
G	768	15		217
H	815	163	22	
I	674	135		191

of treating with PEG and supplementing with urea or sunflower meal were studied as well.

2. MATERIALS AND METHODS

2.1. Apparatus

A continuous culture system, following the design of Miettinen and Setälä [25], has been used. The unit had four fermenter flasks with an effective volume of one litre each, and a vessel to collect the effluents from every fermenter flask. Each fermenter was inoculated with 700 mL of rumen liquor collected from three ruminally cannulated animals and maintained at 39 ºC in a water bath. Artificial saliva [24] was continuously infused at a dilution rate of 0.96 per day and CO_2 was also continuously infused. The contents of the fermenter flasks were constantly homogenised. In order to avoid microbial activity in the effluents, output vessels were maintained in a bath at 3 ºC.

2.2. Diets and experimental procedure

The solid by-product, obtained from the desiccation, extraction and partial pitting of crude olive cake produced from the new two-stage centrifugation technology to obtain olive oil (TSDOC), was used as the base of experimental diets as described in Table I. This by-product was treated with PEG (4,000 MW, 2 or 20 g per 100 g of TSDOC) and supplemented with nitrogen from urea (U) or sunflower meal (SM) (1.5 g N per 100 g OM of TSDOC). In each of the nine incubation runs two fermenters were inoculated with rumen liquor from Granadina goats and another two with rumen liquor from Segureña wethers. The animals received lucerne hay: 167 g crude protein (CP), 451 g neutral detergent fibre (NDF), 366 g acid detergent fibre (ADF) and 17.2 MJ gross energy (GE) per kg dry matter (DM), offered at maintenance level [2, 29]. Each incubation run consisted of a seven-day stabilisation period followed by a four-day sampling period. Fermenters were supplied daily with 21.1 g DM in two equal portions at 08:00 and 16:00 hours. The pH in the vessel was checked twice a day before feeding. Every day, the effluents were removed and their weight and volume were recorded. Every sampling-day, the effluents were kept at –20 ºC and, at the end of the trial, composite sub-samples from 4 sampling days were made and an aliquot was used for NH_3-N and VFA analyses.

The remainder was freeze dried and DM, OM, crude fat and total, amino acid, diaminopimelic acid (DAPA) and N associated with the cellulose of cell wall contents were determined.

2.3. Analytical procedures

Dry matter, ashes, crude fat (CF) and total N (TN) were determined according to the AOAC [4] procedures. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were determined according to the sequential method of van Soest et al. [42] using the ANKOM [3] technology. NDF was assayed with sodium sulphite and without alpha amylase. NDF, ADF and ADL were expressed without residual ashes. Acid-detergent-insoluble N (ADIN) was determined by Kjeldahl analysis of ADF residues. NH_3-N was analysed by the colorimetric method of Weatherburn [43]. Volatile fatty acids were analysed by gas chromatography using centrifuged samples, previously fixed with metaphosphoric acid, following the Jouany [17] method. Total extractable polyphenols (TEP) and total extractable tannins (TET) were determined according to Julkunen-Tiito [18] and Khazaal et al. [20], respectively, using tannic acid as standard. Total extractable (TECT) and total (TCT) condensed tannins were analysed according to Porter et al. [28] methodology and expressed as mg per g DM applying the Makkar and Goodchild [22] formula. Amino acid N (AA-N), including DAPA, was analysed by HPLC using the Pico-Tag method for hydrolysed samples [10].

2.4. Calculations and statistical analyses

The daily output of fermentation products was calculated from the volume of effluents and product concentration in the effluents. Total carbohydrate input was calculated as the difference between OM and crude protein plus crude fat inputs. For total carbohydrate output the amount of hexoses used for VFA production was detracted [12]. Bacterial nitrogen output was calculated as the difference between NAN and ADIN outputs [9]. The efficiency of bacterial N production was expressed as g of bacterial N per kg total digested carbohydrates (DCHO).

The experimental model was the following: $Y = \mu + A_i + N_j + P_k + A \times N_{ij} + A \times P_{ik} + N \times P_{jk} + A \times N \times P_{ijk} + \varepsilon$, where μ is the overall mean, A the animal species, N the nitrogen supplementation, P the PEG treatment and ε the model error.

Data were statistically analysed using the GLM procedure [41]. The PEG, supplementary N and inocula origin effects on the fermentation products concentration and daily output were tested using the variance between two replicates from two vessels by multifactor ANOVA. Treatment means were separated by a t-test for multiple comparisons. The differences between groups were obtained using the Tukey test.

3. RESULTS

The analytical composition of TSDOC and SM is shown in Table II. TSDOC is a lignocellulosic material with a large part of its N associated with lignocellulosic components (70.6 g per 100 g TN). Phenolic compounds were present in TSDOC and in SM, with TSDOC containing the highest amount of TCT (1.38 and 0.39 g per 100 g DM, respectively for TSDOC and SM). The essential amino acid N values were similar for TSDOC and SM (55.2 and 53.3 g AA-N per 100 g TN, respectively). The limiting amino acids in TSDOC seem to be methionine (1.41 g AA-N per 100 g T-AAN) and cysteine (1.04 g AA-N per 100 g T-AAN).

Fermenter pH and OM and total carbohydrate inputs, concentration of total and individual VFA in effluents, VFA and DCHO outputs and efficiency of VFA production are shown in Table III. The values of pH were close to 7.0 in all the fermenters.

Table II. Chemical composition of two stage dried olive cake (TSDOC) and sunflower meal (SM).

	TSDOC	SM
Dry matter, g per 100g fresh matter	87.1	87.1
g per 100 g dry matter		
Organic matter	85.0	93.6
Crude fat	0.13	0.70
Neutral detergent fibre	62.4	56.2
Acid detergent fibre	54.0	35.5
Acid detergent lignin	32.8	12.7
Crude protein (total N x 6.25)	7.88	32.5
Nitrogen attached to ADF (g per 100 g total N)	70.6	10.8
Non-nutritive compounds (mg per g dry matter)		
Total extractable polyphenols	10.5	24.4
Total extractable tannins	9.78	0.60
Total extractable condensed tannins	0.81	0.45
Total condensed tannins	13.8	3.94
g amino acid-N per 100 g total N		
Histidine	3.16	6.15
Arginine	7.74	15.5
Threonine	4.05	2.65
Valine	10.7	7.04
Methionine	1.41	2.55
Isoleucine	4.64	3.31
Leucine	11.4	7.72
Phenylalanine	5.69	4.70
Lysine	6.53	3.64
Aspartic acid	2.67	8.25
Glutamic acid	5.54	13.1
Serine	3.98	4.00
Glycine	6.64	7.36
Alanine	8.56	5.66
Proline	4.80	3.84
Tyrosine	1.62	1.02
Cysteine	1.04	1.99

Average pH was not affected ($P > 0.05$) by the origin of the rumen inoculum, PEG treatment or N supplementation.

Digested carbohydrate output (DCHO, g per day) was not affected ($P > 0.05$) by inoculum origin but it increased ($P < 0.001$) by supplementing with U or SM. Only the highest level of PEG (20 g of PEG per 100 g TSDOC) promoted a significant improvement ($P < 0.001$) of DCHO digestibility.

Table III. Average pH, input of organic matter and total carbohydrates, concentration and output of volatile fatty acids and output of digested carbohydrates in a continuous culture system inoculated with rumen liquor from goats or sheep fed untreated and supplemented with urea (U) or sunflower meal (SM) and/or treated with polyethylene glycol (PEG) two stage dried olive cakes.

	Untreated	Animal Species			Supplementary N				PEG (g per 100 g TSDOC)				Interactions				
		Sheep	Goats	LS	0	U	SM	LS	0	2	20	LS	A×N	A×P	N×P	A×N×P	SEM
pH	7.08	7.02	7.06	NS	7.04	7.10	6.98	NS	7.04	7.05	7.03	NS	NS	NS	NS	NS	0.021
Input of organic matter (g·day^{-1})	14.9	18.1	18.1		16.4	16.9	21.0		16.6	17.0	20.6						
Input of total carbohydrates (g·day^{-1})	13.5	16.1	16.1		15.0	15.5	18.0		14.7	15.1	18.7						
Total VFA (mmol·L^{-1})	15.7	26.5^{b}	24.5^{a}	*	14.0^{a}	22.9^{b}	39.5^{c}	***	23.1^{a}	23.0^{a}	30.4^{b}	***	***	***	***	***	0.369
Composition (mol·100 mol^{-1})																	
Acetic	9.33	58.5^{b}	57.9^{a}	*	55.0^{a}	56.9^{b}	60.3^{b}	***	57.8^{b}	55.1^{a}	61.2^{c}	***	***	***	***	***	0.214
Propionic	3.47	23.4	23.6	NS	24.6^{b}	23.9^{a}	22.9^{a}	***	25.4	25.4	20.6	NS	***	***	***	***	0.085
Iso-butyric	0.105	0.37	0.39	NS	0.76^{b}	0.35^{a}	0.26^{a}	***	0.44^{b}	0.45^{b}	0.28^{a}	***	NS	NS	***	NS	0.001
Butyric	2.59	16.5	16.5	NS	18.7^{b}	18.3^{b}	14.7^{a}	***	15.9^{a}	18.4^{b}	15.5^{a}	**	*	**	**	**	0.084
Iso-valeric	0.089	0.99^{a}	1.17^{b}	*	0.67^{b}	0.35^{a}	1.64^{c}	***	0.44^{a}	0.47^{a}	2.05^{b}	***	**	**	***	***	0.005
Valeric	0.082	0.35	0.38	NS	0.60^{b}	0.33^{a}	0.30^{a}	***	0.40	0.42	0.30	NS	NS		***	NS	0.002
DCHO (g·day^{-1})	5.86	6.99	6.64	NS	5.52^{a}	7.18^{b}	7.15^{b}	***	7.53^{b}	7.49^{b}	5.42^{a}	***	**	NS	***	*	0.175
Total VFA (mmol·day^{-1})	20.5	24.6^{b}	22.8^{a}	*	13.1^{a}	21.1^{b}	36.8^{c}	***	21.8^{a}	21.4^{a}	27.8^{b}	***	***	***	***	***	0.368
mol VFA per kg DCHO	1.88	3.52	3.56	NS	2.51^{a}	3.12^{a}	5.00^{b}	***	3.02^{a}	2.79^{a}	4.82^{b}	***	NS	NS	*	**	0.148

^{a,b,c} Mean values not sharing a common letter are significantly different for each treatment; LS: level of significance; A×N: interaction between animal species and supplementary N; A×P: interaction between animal species and PEG treatment; N×P: interaction between supplementary N and PEG treatment; A×N×P: triple interaction; NS: non significant ($P > 0.05$); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; SEM: standard error of the mean.
VFA: volatile fatty acids; DHCO: total digestible carbohydrates.

The VFA output (mmol per day) was low, and higher ($P < 0.05$) values were obtained with inoculum from sheep, in comparison with goats. The main VFA was acetic acid. Nitrogen supplementation increased ($P < 0.001$) VFA output, having a more important effect with SM than with U. The addition of 20 g PEG per 100 g TSDOC increased ($P < 0.001$) the VFA output. The efficiency of VFA production (mol per kg DCHO) was low and not different ($P > 0.05$) in the fermenters inoculated with rumen liquor from goats or sheep. It increased ($P < 0.001$) with supplementary N, especially from SM, and with the addition of 20 g PEG per 100 g TSDOC.

Daily inputs of total (NI) and amino-acid (AANI) nitrogen and the effects of animal species, N supplementation and PEG addition on ammonia concentration in the effluents and daily outputs of total (NO), lignocellulose bound N (ADIN), amino-acid (AANO) and non-ammonia (NANO) nitrogen are shown in Table IV. ADIN was affected ($P < 0.01$) only by the PEG treatment. The NO obtained in the fermenters with inoculum from goats was higher ($P < 0.05$) than in those inoculated with rumen liquor from sheep but it was not affected ($P > 0.05$) by the PEG treatment. Supplementation with U or SM obviously increased ($P < 0.001$) the total N output. Outputs of AAN and non-ammonia N were not ($P > 0.05$) affected by the inoculum origin but they increased ($P < 0.001$) with N supplementation. Polyethylene glycol treatment did not affect ($P > 0.05$) the AAN output, and only the addition of 20 g PEG increased ($P < 0.05$) NAN output.

The estimated daily output and efficiency of bacterial N production are shown in Table V. Neither bacterial N production nor its efficiency were affected by rumen inoculum origin (Tab. V). Both parameters increased ($P < 0.001$) with N supplementation, the highest values corresponding to TSDOC supplemented with SM. The addition of PEG did not affect ($P > 0.05$) microbial N production, but its efficiency increased ($P < 0.01$) with 20 g PEG per 100 g TSDOC. The DAPA-N output, which is specific of bacteria, indicated that the effects of rumen inoculum and supplementary N origin were similar to those observed for bacterial N. However, PEG treatment significantly increased ($P < 0.001$) the DAPA-N output.

4. DISCUSSION

The chemical composition of TSDOC reflects that it is a lignocellulosic material, rich in structural carbohydrates, above all ADL, and low in available N (ADIN close to 70%). While the cellulose content was similar in TSDOC as in SM, the hemicellulose fraction was 2 times higher in SM.

The average pH values in the fermenters close to 7.0 may be due to a combined effect of continuous buffer infusion and slow TSDOC fermentation, which was also reflected by a low VFA concentration. Therefore, the pH values obtained in the present work were similar to those observed with diets based on shrubs either in vivo [14] or in vitro [26].

The VFA output was lower than the values reported by Molina Alcaide et al. [26] for shrubs and by Carro and Miller [9] for diets rich in structural carbohydrates. The efficiency of VFA production was similar to the values obtained by Molina Alcaide et al. [26]. Supplementation with U or SM stimulated TSDOC fermentation, as shown by the increase of daily VFA production and efficiency (by 1.6 and 2.8 and 1.2 and 2.0 times, respectively). The results obtained with TSDOC agreed with those of other authors, who found a higher stimulating effect on the fermentation when supplementing with NAN, in comparison with ammonia N [9, 16, 26, 31]. The increased acetate production with PEG treatment would indicate an increased fermentation of TSDOC carbohydrates, in agreement with other observations [6, 37]. The acetic:propionic ratio did not increase by the addition

Table IV. Daily inputs of nitrogen (NI) and total amino-acid nitrogen (AANI), ammonia concentration (mg per 100 mL) and daily outputs of total (NO), acid detergent insoluble (ADIN), amino-acid (AANO) and non-ammonia (NANO) nitrogen in a continuous culture system inoculated with rumen liquor from goats or sheep fed untreated and supplemented with urea (U) or sunflower meal (SM) and/or treated with polyethylene glycol (PEG) two stage dried olive cakes.

	Untreated	Animal Species			Supplementary N				PEG (g per 100 g TSDOC)				Interactions				SEM
		Sheep	Goats	LS	0	U	SM	LS	0	2	20	LS	A×N	A×P	N×P	A×N×P	
NI (mg·day^{-1})	219	390	390		219	476	475		390	390	390						
AANI (mg·day^{-1})	196	205	205		196	196	224		205	205	205						
NH_3-N (mg·100 mL^{-1})	0.60	5.42^{a}	9.65^{b}	***	0.82^{a}	14.8^{c}	7.00^{b}	***	8.40^{b}	6.84^{a}	7.37^{ab}	**	***	**	***	***	0.160
NO (mg·day^{-1})	178	347^{a}	384^{b}	*	212^{a}	440^{b}	445^{b}	***	350	357	389	NS	**	NS	NS	**	6.552
ADIN (mg·day^{-1})	107	150	153	NS	138	157	161	NS	138^{a}	142^{a}	176^{b}	**	**	NS	*	***	2.913
AANO (mg·day^{-1})	160	165	178	NS	101^{a}	197^{b}	216^{b}	***	164	172	178	NS	**	NS	***	*	3.389
NANO (mg·day^{-1})	173	291	294	NS	204^{a}	275^{b}	378^{c}	***	271^{a}	283^{a}	323^{b}	*	NS	NS	NS	NS	6.928

[a,b,c] Mean values not sharing a common letter are significantly different for each treatment; LS: level of significance; A×N: interaction between animal species and supplementary N; A×P: interaction between animal species and PEG treatment; N×P: interaction between supplementary N and PEG treatment; A×N×P: triple interaction; NS: non significant ($P > 0.05$); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; SEM: standard error of the mean.

Table V. Estimated daily outputs (mg per day) and efficiency (g per kg DCHO) of bacterial nitrogen production in a continuous culture system inoculated with rumen liquor from goats or sheep, fed two stage dried olive cakes untreated and supplemented with urea (U) or sunflower meal (SM) and/or treated with polyethylene glycol (PEG).

	Untreated	Animal Species			Supplementary N				PEG (g per 100 g TSDOC)				Interactions				
		Sheep	Goats	LS	0	U	SM	LS	0	2	20	LS	A×N	A×P	N×P	A×N×P	SEM
mg bacterial N per day	66.0	141	141	NS	66.0^{a}	118^{b}	217^{c}	***	133	141	147	NS	NS	NS	NS	NS	3.610
g bacterial N per kg DCHO	11.3	20.2	21.2	NS	12.0^{a}	16.4^{b}	30.3^{c}	***	17.7^{a}	18.8^{a}	27.1^{b}	**	NS	NS	NS	NS	0.962
Diaminopimelic acid-N output (mg per day)	1.14	2.01	1.98	NS	1.37^{a}	2.43^{b}	2.51^{c}	***	1.82^{a}	1.17^{a}	2.45^{b}	***	NS	NS	**	NS	0.116

^{a,b,c} Mean values not sharing a common letter are significantly different for each treatment; LS: level of significance; A×N: interaction between animal species and supplementary N; A×P: interaction between animal species and PEG treatment; N×P: interaction between supplementary N and PEG treatment; A×N×P: triple interaction; NS: non significant ($P > 0.05$); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; SEM: standard error of the mean; DCHO: total digestible carbohydrates.

of both N sources (that is urea and sunflower meal). The values obtained were similar to those observed by Karunanandaa and Varga [19] and Ranilla et al. [31] with fibre from sugarbeet pulp and different forms and amounts of N. However, the values obtained by Ranilla et al. [31], using fibre from barley straw, were lower than those obtained with TSDOC.

The addition of PEG increased total VFA output and its efficiency. This trend could be the result of high carbohydrate utilisation, as lower DCHO content in the effluents from fermenters added with 20 g PEG per 100 g TSODC reflected.

Branched-chain VFA result from the degradation of branched-chain amino acids, and the reduction of protein degradation in the rumen has been associated with these VFA concentration decreases [8]. Although valine and leucine values in TSDOC were higher than in the shrubs used by Molina Alcaide et al. [26], the iso-butyric and iso-valeric values found in the effluents were lower. Only supplementation with SM increased iso-valeric concentration. On the contrary, only the addition of 20 g PEG increased iso-valeric production, perhaps due to a limitation of lower amounts of PEG in overcoming the deleterious effect of tannins. This fact would also suggest a microbial origin of the branched-chain fatty acids found in the effluents.

The ammonia concentration in the effluents was low (0.66 mg per 100 mL, respectively). It was lower than the recommended level for microbial growth [33, 35], although reported values are variable [5, 7, 11, 35]. Minimal ammonia concentration may depend on the substrate availability [13] or on the microorganism species taking part in its degradation. Losses of N in continuous culture systems [11] could also explain low ammonia concentration and, in this experiment, average recoveries of N were close to 0.80. Increased ammonia concentration and daily VFA and bacterial N production were obtained by supplementing either with U or SM. This could indicate a limited availability of TSDOC N for carbohydrate fermentation. The high ammonia concentration found, above all, when supplementing with U, seems to indicate that N supply was higher than required, which could also explain the reduced effect of PEG addition.

Total AA-N outputs in fermenters fed unsupplemented TSDOC or this by-product supplemented with U were slightly higher than the values reported by Molina Alcaide et al. [26] with shrubs. If TSDOC and shrubs supplemented with SM are compared, total AA-N flow was higher in the fermenters fed shrubs, indicating that this N form would be less appropriate for TSDOC than it is for shrubs. However, a significant increase of AA-N output when TSDOC was supplemented with U indicates an incorporation of ammoniacal nitrogen into microbial protein.

Estimated bacterial protein production promoted by TSDOC ruminal fermentation is similar to that found with poor feedstuffs such as shrubs [26] or cereal straw [19], but it was higher than that obtained with diets rich in structural carbohydrates [9]. Calculated values for microbial protein synthesis efficiency were similar to those obtained in fermenters fed shrubs [26]. The higher efficiency promoted by SM supplementation in comparison with unsupplemented TSDOC or supplemented with U, would be due to the addition not only of a better quality N but also of easily degradable carbohydrates from SM, which could be a limiting factor for bacterial development.

The significant improvement of bacterial production efficiency and DAPA-N output by PEG addition is contrary to the observations of Makkar [21] who found that 2 to 3% of tannins in the diet reduced fibre degrading bacteria, while the efficiency of microbial protein synthesis was not affected. This effect of PEG in our case could be, to some degree, affected by the significant ($P < 0.01$) supplementary N × PEG addition interaction.

Interspecies differences lack concerning AGV and bacterial N production and efficiencies could be ascribed to the standard diet (alfalfa hay) supplied to inoculum donors. Animals seem to require tannin rich diets in order to develop a specific microbial ruminal population and, as a consequence, to show differences between goats and sheep [36].

5. CONCLUSIONS

Volatile fatty acid production from TSDOC fermentation was low and mainly acetic. The efficiency of VFA production was low as well. Protein degradation of TSDOC was also low, but its promoted microbial growth was similar to the one from other poor quality feedstuffs. Quantitative and qualitative characteristics of TSDOC fermentation were only slightly affected by the origin (goats vs. sheep) of inoculum used in the fermenters. The response to N supplementation indicates a deficit of N and subsequent depression of carbohydrate fermentation; N from SM gave better results than U when supplementing TSDOC. The effect of tannins on TSDOC fermentation seems to be relevant since PEG addition improved protein and energy availability.

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