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Belanche A., Fuente G. de la, Guada J.A., Balcells J.

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Effect of type roughage and level of barley supplementation on digestibility, rumen fermentation and microbial-N yield

A. Belanche*, G. De la Fuente*, J.A. Guada* and J. Balcells**

*Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, C/ Miguel Servet 177, 50013 Zaragoza (Spain)
**Departament de Producció Animal, ETSEA, Av. Alcalde Rovira Roure 191, 25198 Lleida (Spain)
balcells@prodan.udl.cat

Abstract. Four cannulated ewes were used in a factorial (2 x 3) design to study the effect of two sources of roughage (alfalfa hay *vs* ammonium-treated straw, UTS) and three levels of ground barley grain supplementation (F:C ratio: 100:0; 66:33; 33:66. respectively) on rumen digestibility, fermentation pattern and microbial N yield. Diets were restricted to maintenance level (1.6 Mcal ME/d). Rumen (0.40 *vs* 0.34; P=0.07) and total tract DM digestibility were higher (0.77 *vs* 0.68; P<0.001) when ewes were fed with alfalfa diets and digestibility increased linearly with concentrate supply (P<0.001). Alfalfa diets also promoted higher rumen ammonia (31 *vs* 20 mg $N\text{-}NH_3$/100ml; P=0.003) and VFA concentrations (144 *vs* 82 mmol/l; P<0.001). Among levels of barley supply only the highest (F:C. 33:66) depressed significantly pH and acetate proportion (P=0.008). Protozoa counts increased significantly (P<0.05) with moderated barley supplementation ($3.2 \cdot 10^5$ *vs* $8.2 \cdot 10^5$ *vs* $7.6 \cdot 10^5$ cells/ml). The highest and the lowest duodenal N flow corresponded to un-supplemented alfalfa and un-supplemented straw diets as a result of a significant (P<0.001) interaction (forage x supplementation). Moreover, rumen microbial yield was maximal when animals received non-supplemented alfalfa (9.0, 12.1 and 8.0 g/d using ^{15}N, purine bases (PB) and DNA sequences, respectively). Using specific DNA sequences it was possible to distinguish between bacterial and protozoal N flow in duodenal digesta. Protozoal N in duodenal digesta ranged between 2.4 and 8.3 % of total microbial flow and peaked at the highest level of grain supply (F:C 33:66). However, the possibility for DNA degradation by gastric digestion could not be ruled out. Barley supplementation promoted a linear decrease in microbial protein synthesis efficiency (g/kg OM digested in the rumen) using PB and DNA as microbial markers (37 *vs* 27 *vs* 23; P<0.05).

Keywords. Rumen microbial – Yield-microbial markers – Purine bases – qPCR – DNA.

Effet de différent types de fourrage et d'orge sur la digestibilité, la fermentation ruminale et la production d'azote microbien

Résumé. *Quatre brebis portant une canule du rumen ont été utilisées dans une analyse factorielle (2x3) afin d'étudier l'effet de 2 types de fourrage (luzerne* vs *paille traitée avec du NH3) et de 3 niveaux d'orge en grain supplémentée (F:C 100:0; 66:33; 33:66.) sur la digestibilité ruminal et des paramètres de fermentation. Ces deux régimes ont été limités au niveau d'entretien. La digestibilité de la matière sèche (DM) dans le rumen et sur l'ensemble du tube digestive est supérieur dans les brebis nourries avec le régime à base de luzerne et augmente linéairement avec l'apport du concentré. Les régimes de luzerne ont aussi produit des concentrations supérieures en ammonium et VFA. Entre les niveaux d'orge supplémentés, seulement le plus haut a diminué significativement le pH et la proportion d'acétique. La population des protozoaires est significativement élevée avec la supplementation modérée d'orge. Le plus haut et le plus bas apport de N et le flux duodénal correspondent au régime de luzerne et de paille non supplémentés, respectivement, du fait d'un effet significatif sur l'interaction (fourrage x suppléments). Le flux de N microbien à travers le rumen a été supérieur si les animaux ont reçu du luzerne sans suppléments, du fait de la valorisation de MN avec l'usage de N, PB et ADN comme marqueurs microbiens et l'utilisation des bactéries associés à la phase liquide comme référence. Néanmoins, lors de l'emploi des bactéries associées à des particules comme référence, les différences n'ont pas été statistiquement significatives. En utilisant des séquences spécifiques d'ADN, il a été possible de différencier les flux de N bactérien et de N protozoaire dans la digestion duodénal. Le N proto-*

zoaire dans la digestion duodénal varie entre 2,4 et 8,3% du totale MN et arrive au maximum dans les régimes de paille supplémentée, si l'on considère des bactéries associées à la phase liquide. Mais, une dégradation de l'ADN ne peut être rejetée. En termes du rendement de synthèse de protéine microbienne (g/kg de matière organique ruminal dégradée) il n'y a pas de différences importantes entre les valeurs des différents marqueurs microbiens utilisés bien qu'entre les traitements, le majeur rendement a eu lieu lorsque les animaux ont reçu de la luzerne comme le seul régime.

Mots-clés. *Proteine microbienne – Marqueur microbien – Base purinique – q-PCR – ADN.*

I – Introduction

In the Mediterranean region, scarcity of roughage combined with the increase in demand for livestock production has driven ruminant production toward increasingly intensive feeding systems whose optimisation represents an important challenge for ruminant nutrition studies. Grain has replaced roughage as the main source of fermentable carbohydrates in order to improve production efficiency. However, the feeding of concentrate rations may lead to gastric dysfunction, such as acidosis, where the microbiota specialized in the fermentation of fibre is transformed into a microbial ecosystem adapted for fermentation of non-structural carbohydrates (NSC). In theory, dietary energy is used more efficiently with NSC-diets; feeding starch increases propionate production and reduces the maintenance cost of rumen microbial populations (Czerkawski, 1978). However, the effect of NSC-based diets on both, microbial yield and efficiency is not yet clear. Uncertainties are due mainly to the inability to quantify accurately microbial protein yield in the rumen. The variability of the estimation of microbial synthesis using different microbial markers and their ability to discriminate between microbial and non-microbial N flow in the chymus, may be regarded as the most critical aspect to this study.

The aim of the present study was to analyse the effect of the different grain supplements on two basal diets (alfalfa hay or urea-treated barley straw, UTS) on rumen microbial yield estimated using purine bases (PB) and specific DNA sequences (DS) as internal markers and ^{15}N as an external microbial marker. Rumen fermentation patterns and digestibility were also studied.

II – Materials and methods

Four adult, dry, non-pregnant Rasa Aragonesa ewes (48 SD 6 kg live weight) with surgically implanted ruminal and duodenal cannulas were undergone successively to six 15 day periods. During each period individual animals were randomly allocated one diet following a factorial (2x3) design; two types of roughage (alfalfa hay *vs* urea treated straw, UTS) and three levels of ground barley grain supply (F:C ratio: 100:0; 66:33; 33:66. respectively) were studied. To avoid carry over effects between roughages, animals were fed initially with diets based on alfalfa hay (periods 1, 2 and 3) and then with UTS diets (periods 4, 5 and 6). Animals were handled according to the EU criteria for care and use of animals in research and the experimental protocols were approved by the Ethical Committee of the University of Zaragoza (Ref. PI13/05).

Diets were formulated to fulfil maintenance requirements (1.01 Mcal ME/Kg $LW^{0.75}$, AFRC. 1993). After seven days of adaptation to the diet, animals were placed in metabolic cages equipped with automatic feeders and free access to fresh water. On the day prior to be used, barley straw was watered with urea 0.4 l/kg fresh matter at 7.5% w/v. Complete diets were offered in eight daily meals and intake and total tract digestibility were recorded during the following five days. From day 9, Cr-EDTA (208 mg/d) and ^{15}N (1.6 mg/g N ingested) were continuously infused in the rumen and used as liquid-flow and microbial markers respectively. After four days of infusion,

nine duodenal digesta samples (~50 g) were taken (every two hours starting at 8 h am) during days 13 and 14. Homogenized duodenal samples were immediately frozen at –20ºC, pooled and freeze-dried and used to determine rumen degradability and microbial yield, while a subsample (~0.3 g) was frozen in liquid-N for DNA extraction. The last day (15 d) rumen content was sampled at 0, 1 and 3 hours after feeding and used to describe the fermentation pattern. Rumen contents (500 g) were filtered through two layers of cheesecloth; the filtrate was subsampled to measure ammonia, volatile fatty acids (VFA) and to determine protozoal numbers. The rest of the rumen fluid was divided into two fractions, to isolate liquid-associated bacteria (LAB) and protozoa (LAP) following Pérez *et al.* (1995) and Sylvester *et al*. (2004) procedures, respectively. Microbial extracts were frozen and freeze-dried for storage.

Dry matter (DM) content in both feed and faeces was determined by drying samples (105ºC) to a constant weight and organic matter (OM) by combustion at 550ºC for 8 h in a muffle furnace. NDF and ADF concentrations were determined by the procedures proposed by Van Soest *et al*. (1991). Total N in food, refusals and microbial extracts was determined by the Kjeldahl method using Selenium as a catalyst. Non-ammonia N (NAN) in duodenal digesta was determined after removing the ammonia (Firkins *et al*., 1992). NH_3-N concentration in rumen fluid was estimated following the colorimetric procedure proposed by Chaney and Marbach (1962). Volatile fatty acid (VFA) concentration in deproteinised rumen fluid was determined by gas liquid chromatography (Jouany and Senaud, 1982). Alkanes, used as a solid-associated flow marker, were extracted from diet, refusal and duodenal samples (Mayes *et al*., 1988) and were analysed as described by Valiente *et al*. (2003). PB in digesta and microbes extracts was determined using the method proposed by Balcells *et al.* (1992) with the modification proposed by Martín-Orúe *et al.* (1996). The isotope abundance of ^{15}N was quantified using a mass spectrophotometer (VG PRISM II. IRMS, Carlo Erba. Milan, Italy). Protozoal cells were microscopically counted (Dehority, 1993) using Lee *et al*. (1985) classification protocol.

DNA was extracted in duplicate from frozen samples using the QIAamp® DNA Stool Mini Kit (Qiagen Ltd. Carwley, West Sussex, UK) following the manufacturer's instructions. Bacterial and protozoal DS concentration was determined in duodenal samples by a quantitative PCR following the protocols described by Maeda *et al*. (2003) and Sylvester *et al*. (2004) respectively. Extracted DNA (2 µl) was added to amplification reaction (25 µl total volume) containing 25 pmol of each primer, 12.5 µl of Platinum® SYBR® Green qPCR SuperMix-UDG and 0.5 µl of ROX Reference Dye (Invitrogen™). Cycling conditions for bacterial DS quantification were 95ºC for 10 min followed by 30 cycles of 95ºC for 15 s, 61ºC for 30 s and 72ºC for 30 s, while for protozoal DS quantification the annealing temperature was 55ºC for 20 s. DNA sequence concentration in bacterial and protozoal extracts was assessed with a spectrophotometer, corrected in respect to their cross contamination and finally used as standard for the qPCR quantification of the bacterial and protozoal DS in duodenal samples respectively.

Post-ruminal flows were estimated using the double-marker method (Faichney, 1975) assuming a complete recovery of Cr-EDTA and dietary C_{31} in the duodenum (Keli *et al*., 2008). The microbial N (MN) contribution to abomasal NAN was estimated from ^{15}N enrichment PB/N ratio and DS/N ratio in duodenal digesta and microbial extracts.

Data were analyzed by ANOVA as a 2x3 factorial design considering two forages (alfalfa hay *vs* UTS) and three supplementation levels (F:C ratio: 100:0; 66:33; 33:66) as fixed factors and animals as block. All analyses were made using the GLM procedure in the SAS statistical package, version 8.01 (2000) and the least significant difference test was used for comparing means. Differences among means with $P<0.05$ were accepted as statistically significant and those with $0.05<P<0.10$ as statistical tendencies.

III – Results and discussion

All sheep remained in good health throughout the experiment; diets were accepted and animals remained at a similar intake level (414 g digestible OM/d). Refusals were always less than 10% of offered diet, with the exception of the sheep receiving non-supplemented treated-straw (274 g digestible OM/d).

Treatment of straw improved its crude protein content (from 3.6% to 11.9%) and digestibility (Fondevila and Dehority, 1994), however, the improvement was not sufficient to reach the nutritional quality of forage such as alfalfa hay. Therefore, sheep eating alfalfa showed a higher total tract digestibility of OM (79% *vs* 69%, P<0.001), NDF (66% *vs* 59%, P=0.055) and ADF (66% *vs* 53%, P=0.002), although these differences between forages did not reach the statistical significance in terms of rumen degradability due to the high variability inherent in the estimation procedure. Ground barley grain is an easily fermentable carbohydrate source for rumen microbes and caused a linear increment in rumen OM degradability (40% *vs* 51% *vs* 56%, P=0.003) and total tract digestibility (66% *vs* 74% *vs* 81%, P<0.001).

Barley supplementation and type of forage caused changes in the fermentation pattern (Table 1). There was a direct relationship between digestible OM intake and the rumen VFA concentration; while the relationship was opposite with rumen pH. These differences explain the greater VFA concentration (144 mM *vs* 82 mM, P<0.001) and lower pH (6.23 *vs* 6.47, P=0.067) observed in alfalfa compared to UTS diets. Rumen pH (6.58 *vs* 6.42 *vs* 6.05, P=0.008) and acetate proportion (77% *vs* 75% *vs* 70%, P<0.001) reduced linearly with respect to barley supplementation whilst butyrate proportion (5.2% *vs* 8.0% *vs* 9.2%, P<0.001) increased. Several authors (Jaakkola and Huhtanen, 1993; Koenig *et al*., 2000) suggested that the increase in butyrate would be related to the increase in protozoal and their tropism for the starch sources and our findings seem to confirm this hypothesis ($3{\cdot}10^5$ *vs* $8{\cdot}10^5$ *vs* $8{\cdot}10^5$ cells/ml, P=0.042 for 0, 33 and 66% supplementation). Type of forage did not alter protozoa counts but it did modify the structure of the protozoal community (Harrison and McAllan, 1980). Alfalfa supplementation reduced small protozoa numbers (*Entodinium*, P<0.001) but significantly increased the abundance of larger protozoa, such as *Diplodinium*, *Ophryoscolex*, *Isotricha* and *Dasytricha*. A greater proteolytic/predation capability of *Dasytricha* compared to *Entodinium* has recently been investigated (de la Fuente *et al*., 2009). High proteolytic activity and N turnover in the rumen under alfalfa diets would explain the greater rumen ammonia-N concentration (31 mg/dL *vs* 20 mg/dl, P=0.003) and specific microbial end-products (iso-butyrate, valerate and iso-valerate) of amino-acid degradation in these diets.

Post-ruminal NAN flow reflected N intake and was higher when animals were fed with alfalfa than with UTS (13.53 g/d *vs* 9.1 g/d, P<0.001, Table 2) representing on average 88 SD 2.6% of the N ingested. Animals fed with alfalfa hay showed an apparently higher yield of rumen microbial N, although differences only reach statistical significance when data were estimated using PB as microbial marker (8.6 g/d *vs* 6.13g/d for alfalfa and UTS diets, P=0.015). The effect of barley supplementation on rumen microbial yield was influenced by the type of roughage. Microbial production was at its highest when alfalfa was provided as the whole diet, however, microbial production decreased with barley supplementation (Table 2). This effect was consistent and independent of the microbial marker used. The rumen microbial population in the animals fed with UTS showed a different behaviour and supplementation with barley improved the microbial N flow, either linearly in those values derived from ^{15}N, or quadratically when PB or DS were used as microbial markers; in both cases the optimum level was reached at 33% of barley supplementation.

Theoretically, alfalfa hay, as most of the leguminous roughages, has a limited amount of rumen fermentable energy in relation to its protein content. Thus, microbial yield in alfalfa diets should be increased with a fermentable energy supplement (i.e. barley grain). However, our data did not support this hypothesis and the change in fermentation conditions with barley supplementation

Table 1. Effect of forage type and barley supplementation on digestibility and rumen fermentation

Forage	Alfalfa hay			Treated-Straw†			S.E.M	Signification		
Barley Supply	**0%**	**33%**	**66%**	**0%**	**33%**	**66%**	*n=4*	**Forage**	**Supply**	**F x S**
Intake (g/d)										
OM	636^{a}	474^{b}	460^{b}	452^{b}	577^{a}	586^{a}	30.6	0.559	0.762	<0.001
Apparent digestibility (%)										
OM	72	79	86	61	69	77	2.69	<0.001	<0.001	0.947
NDF	64	64	72	60	59	57	4.28	0.055	0.734	0.377
ADF	64	65	70	55	54	49	4.53	0.002	0.985	0.417
Rumen apparent digestibility (%)										
OM	40	55	57	41	46	54	2.13	0.247	0.003	0.409
N	15	22	1.9	13	17	5.9	6.24	0.840	0.071	0.767
Rumen fermentation										
pH	6.37	6.29	6.02	6.78	6.54	6.07	0.15	0.067	0.008	0.477
$N\text{-}NH_3$(mg/100 ml)	30	33	31	19	24	16	3.92	0.003	0.374	0.742
Total VFA (mmol/l)	137	148	147	70	94	81	15.9	<0.001	0.558	0.907
Proportion (%)										
Acetate	75^{ab}	74^{ab}	72^{bc}	79^{a}	75^{ab}	68^{c}	1.66	0.912	0.004	0.047
Propionate	17	14	17	16	16	20	1.49	0.235	0.154	0.345
Butyrate	5.7^{cd}	8.8^{ab}	8.1^{b}	4.7^{d}	7.3^{bc}	10^{a}	0.63	0.822	<0.001	0.025
Iso-butyrate	0.9	0.9	0.8	0.1	0.3	0.4	0.10	<0.001	0.608	0.113
Valerate	1.1^{a}	0.9^{ab}	1.1^{a}	0.2^{c}	0.6^{b}	0.9^{ab}	0.16	<0.001	0.038	0.032
Iso-valerate	0.9	0.9	0.7	0.4	0.6	0.9	0.12	0.067	0.365	0.064
Protozoal cells (x10^5/ml)	3.8	10	5.6	2.6	6.5	9.7	1.94	0.911	0.042	0.170
Proportion (%)										
Entodinium	86	86	83	97	94	96	2.53	<0.001	0.681	0.542
Diplodinium	1.7	1.9	1.9	0.6	1.2	0.6	0.63	0.067	0.867	0.906
Ophryoscolex	1.6	3.5	6.1	0.2	0.4	1.0	1.16	0.004	0.105	0.310
Isotricha	2.4	2.3	2.1	0.1	0.9	0.4	0.45	<0.001	0.628	0.662
Dashytricha	8.2	6.2	7.4	1.9	3.5	1.7	1.18	<0.001	0.911	0.310

Means in a row with unlike superscripts are significantly different (interaction F x S; P<0.05).

† Barley straw treated with 35g urea / kg DM.

seems to lead to a negative effect, either limiting rumen microbial growth or alternatively by reducing the efficiency of microbial turnover. Conversely, animals fed with UTS diet showed a higher microbial protein yield when they were supplemented with barley and this improvement peaked with moderate levels of supplementation (F:C ratio 67:33).

Critical to the present study was the precision of microbial marker used; ^{15}N of PB provided consistent and similar measurements of microbial yield, not only in absolute terms but also describing the differences between the experimental treatments. The slight overestimation associated with PB has been conventionally attributed to the presence of non-microbial purines arising either from endogenous secretions (González-Ronquillo *et al*., 2003) or from dietary PB that are able to by-pass rumen fermentation (Askar *et al*., 2005). Specific DS enables the discrimination of microbial species in the chymus (Bergen, 2004) and furthermore can quantify the contribution of non-bacterial species to the host digestive system (Sylvester *et al*., 2004). However, estimates of rumen microbial yield derived from DS were much lower (average 4.0 g MN/d) than those obtained with conventional markers (average 7.0 and 7.4 for ^{15}N and PB). Such an underestimation might be attributed to DS degradation through abomasal digestion. Belanche *et al*. (2010) demonstrated in an *in vitro* simulation that protozoa-DS were sensitive to gastric digestion (around 25% of specific protozoa-DS were digested), nevertheless this author reported that most of the specific bacterial-DS (up to 90%) were able to maintain their molecular integrity throughout gastric digestion. In this case, further research would be necessary. If a fraction of protozoa-DS is digested during the gastric transit, then absolute measurements of rumen protozoa yield based on DS persistence would be underestimated. However, the results presented in this study provide some evidence of the relative measures among treatments. No differences were detected between roughage sources in the protozoa-N flow but, in relation to grain supply, the effect was similar to the total MN yield, decreasing in alfalfa and improving in UTS diets (Interaction Forage x Supplementation, $P<0.05$). However, in relative terms the protozoal N contribution to total MN was significant and increased linearly with barley supplementation (2.5% *vs* 3.8% *vs* 6.5%, $P<0.05$) reflecting the abundance observed in rumen liquor (Table 1).

In terms of efficiency of microbial protein synthesis (gMN/kg OM apparently digested in the rumen – OMDR), no differences were observed between forages (Table 2). At maintenance level, the efficiency was similar regardless of the origin of the digestible OM (high or poor quality roughages) and even the type of N source (mainly protein-N in alfalfa hay *vs* mainly NAN in UTS). These observations fit perfectly with the AFRC (1993) principle based on the metabolizable energy ingestion, although it did not agree with the theory that proposes there are differences in the microbial N yield efficiency depending of the N source. Some amino-acids and peptides are considered as growing factors for rumen microorganisms (Maeng *et al.*, 1976) and most authors agree that rumen microorganisms have some requirement for protein-N (Nocek and Russell, 1988). However, our data suggest that these requirements can be provided by microbial N recycling when animals are fed at maintenance level.

Regardless of the type of forage used, increasing the proportion of easily fermentable carbohydrates (i.e. ground barley grain) in the diet led to a progressive decrease of the microbial synthesis efficiency, an effect that was shown as statistically significant when PB ($P=0.012$) and DS ($P=0.04$) were used as microbial markers. Low efficiencies of microbial synthesis associated with concentrated diets have been widely described (Askar *et al*., 2008). However, it remains unclear whether this could be attributed to higher digestible OM intake or to the reduction of the normal rate of microbial protein synthesis. In describing this effect, several terms like "overflow metabolism", "slip reactions", "uncoupling" and "energy spilling" have been used to explain the hypothetical energy dissipation caused by futile cycles of potassium, ammonium, or protons through the cell membrane when they are fed with a high concentrate diets (Van Kessel and Russell, 1996).

Table 2. Effect of forage type and barley supplementation on microbial N flow and efficiency of microbial synthesis estimated with different microbial markers

Forage	Alfalfa hay			Treated-Straw†			S.E.M	Signification		
Barley Supply	**0%**	**33%**	**66%**	**0%**	**33%**	**66%**	*n=4*	**Forage**	**Supply**	**F x S**
Intake N (g/d)	22^{a}	14^{b}	11^{c}	9.0^{c}	11^{c}	11^{c}	0.75	<0.001	<0.001	<0.001
Duodenal flow (g/d)										
Non-ammonia N	18.9^{a}	10.8^{b}	10.9^{b}	7.8^{c}	9.2^{bc}	10.3^{bc}	1.00	<0.001	0.011	<0.001
Microbial-N flow††										
^{15}N	9.0^{a}	5.8^{bc}	6.4^{abc}	5.1^{c}	6.8^{abc}	8.7^{ab}	1.00	0.811	0.445	0.017
PB	12.1^{a}	8.0^{b}	5.6^{b}	5.4^{b}	7.1^{b}	5.9^{b}	1.08	0.015	0.043	0.012
DS	8.0^{a}	3.0^{b}	2.9^{b}	2.5^{b}	5.3^{ab}	2.1^{b}	1.27	0.219	0.128	0.025
DS-Bacterial	7.8^{a}	2.9^{b}	2.8^{b}	2.4^{b}	5.1^{ab}	1.9^{b}	1.26	0.208	0.120	0.027
DS-Protozoal	0.16^{ab}	0.08^{b}	0.09^{ab}	0.07^{b}	0.15^{ab}	0.18^{a}	1.03	0.380	0.702	0.041
EMS (g MN/kg OMDR)										
^{15}N	36	23	24	30	27	28	5.2	0.844	0.282	0.569
PB	48	30	22	32	28	19	5.7	0.159	0.012	0.395
DS	32	11	11	14	20	7	4.9	0.284	0.040	0.049

Abreviations: purine bases (PB), DNA sequences (DS), efficiency of microbial synthesis (EMS), microbial-N((MN),OM apparently digested in the rumen (OMDR).
Means in a row with unlike superscripts are different (interaction F x S; P<0.05).

† Barley straw treated with 35g urea / kg DM.

†† Microbial-N flow was determined considering liquid-associated bacteria or protozoa as microbial reference extracts.

IV – Conclusions

The effect of barley supplementation on microbial N yield (g MN/d) was affected by type of roughage. Supplementation increased the microbial protein yield using low-quality roughages (i.e. ammonium treated straw), whereas the opposite effect was true when animals were fed with high-quality roughages (i.e. alfalfa hay). Efficiency of microbial protein synthesis (g MN/ Kg OMDR) was reduced when roughage was replaced by concentrate as digestible carbohydrate source.

References

AFRC 1993. *Agricultural and Food Research Council: Energy and protein requirements of ruminant.* An advisory manual prepared by the ARC technical Committee on response to nutrients. CAB International, Wallingford. Oxon, U.K.

Askar A.R., Guada J.A., Balcells J., de Vega A. and Castrillo C., 2005. Validation of use of purine bases as a microbial marker by N-15 labelling in growing lambs given high-concentrate diets: effects of grain processing, animal age and digesta sampling site. In: *Animal Science,* 81, p. 57-65.

Askar A.R., Guada J.A., González J.M., de Vega A. and Fondevila M., 2008. Rumen digestion and microbial protein synthesis by growing lambs fed high-concentrate diets: Effects of cereal processing and animal age. In: *Animal Feed Science and Technology,* 142, p. 292-305.

Balcells J., Guada J.A., Peiró J.M. and Parker D.S., 1992. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography. In: *Journal of Chromatography, Biomedical Applications,* 575, p. 153-157.

Belanche A., Balcells J., Calleja L. and Erroa R., 2010. Use of quantitative real-time PCR to assess the in vitro survival of specific DNA gene sequences of rumen microbes under simulated abomasal conditions. In: *Journal of Animal Physiology and Animal Nutrition,* 94, p. 204-211.

Bergen W.G., 2004. Quantitative determination of rumen ciliate protozoal biomass with real-time PCR. In: *Journal of Nutrition,* 134, p. 3223-3224.

Chaney A.L. and Marbach E.P., 1962. Modified reagents for determination of urea and ammonia. In: *Clinical Chemistry,* 8, p. 130-132.

Czerkawski J.W., 1978. Reassessment of efficiency of synthesis of microbial matter in the rumen. In: *Journal of Dairy Science,* 61, p. 1261-1273.

De la Fuente G., Belanche A., Balcells J. and Fondevila M., 2009. Effect of the rumen environment and type of supplemented nitrogen on the predation of rumen bacteria by protozoa in vitro. In: *Ruminant physiology. Digestion, metabolism, and effects of nutrition on reproduction and welfare,* Y. Chilliard, F. Glasser, Y. Faulconnier, F. Bocquier, I. Veissier and M. Doreau (eds.). Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 154-155.

Dehority B.A., 1993. *Laboratory manual for classification and morphology of ruminal ciliate protozoa*. CRC Press, Boca Raton, FL.

Faichney G.J., 1975. The use of markers to partition digestion within the gastrointestinal tract of ruminants. In: *Digestion and metabolism in the ruminant,* I.W. McDonald and A.C.I. Warner (eds.). University of New England Publishing Unit, Armidale, NSW, Australia, p. 227-291.

Firkins J.L., Weiss W.P. and Piwonka E.J., 1992. Quantification of intraruminal recycling of microbial nitrogen using nitrogen-15. In: *Journal of Animal Science,* 70, p. 3223-3233.

Fondevila M. and Dehority B.A., 1994. Degradation and utilization of forage hemicellulose by rumen bacteria, singly in coculture or added sequentially. In: *Journal of Applied Bacteriology,* 77, p. 541-548.

Gonzalez-Ronquillo M., Balcells J., Guada J.A. and Vicente F., 2003. Purine derivative excretion in dairy cows: endogenous excretion and the effect of exogenous nucleic acid supply. In: *Journal of Dairy Science,* 86, p. 1282-1291.

Harrison D.G. and McAllan A.B., 1980. Factors affecting microbial growth yields in the reticulo-rumen. In: Ruckebusch, Y. and Thivend, P. (eds.), *Digestive physiology and metabolism in ruminants* (Proc. 5th Internat. Symp., Clermont-Ferrand, 3-7 Sept. 1979).

Jaakkola S. and Huhtanen P., 1993. The effects of forage preservation method and proportion of concentrate on nitrogen digestion and ruminal fermentation in cattle. In: *Grass and Forage Sciences,* 48, p. 146-154.

Jouany J.P. and Senaud J., 1982. Effect of rumen ciliates on the digestion of different carbohydrates in sheep. I. - Utilization of cell wall carbohydrates (cellulose and hemicelluloses) and of starch. In: *Reproduction, Nutrition, Development,* 22, p. 735-752.

Keli A., Andueza D., De Vega A. and Guada J.A., 2008. Validation of the *n*-alkanes and NIRS techniques to estimate intake, digestibility and diet composition in sheep fed mixed lucerne: ryegrass diets. In: *Livestock Science,* 119, p. 42-54.

Koenig K.M., Newbold C.J., McIntosh F.M. and Rode L.M., 2000. Effects of protozoa on bacterial nitrogen recycling in the rumen. In: *Journal of Animal Science,* 78, p. 2431-2445.

Lee J.J., Hutner S.H. and Bovee E.C., 1985. *Illustrated guide to the protozoa.* Society of Protozoologists, Lawrence, Kansas. USA.

Maeda H., Fujimoto C., Haruki Y., Maeda T., Kokeguchi S., Petelin M., Arai H., Tanimoto I., Nishimura F. and Takashiba S., 2003. Quantitative real-time PCR using TaqMan and SYBR Green for *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia*, tetQ gene and total bacteria. In: *FEMS Immunol Med Microbiol,* 39, p. 81-86.

Maeng W.J., Vannevel C.J., Baldwin R.L. and Morris J.G., 1976. Rumen microbial growth rates and yields. Effect of amino-acids and protein. In: *Journal of Dairy Science,* 59, p. 68-79.

Martín Orúe S.M., Dapoza C., Balcells J. and Castrillo C., 1996. Purine derivatives excretion in lactating ewes fed straw diets with different levels of fish meal. In: *Animal Feed Science and Technology,* 63, p. 341-346.

Mayes R.W., Lamb C.S. and Colgrove P.M., 1988. Digestion and metabolism of dosed even-chain and herbage odd-chain *n*-alkanes in sheep. In: *Proceedings of the 12th General Meeting of the European Grassland Federation,* Dublin, Ireland, July 4-7, 1988.

Nocek J.E. and Russell J.B., 1988. Protein and energy as an integrated system – Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk-production. In: *Journal of Dairy Science,* 71, p. 2070-2107.

Pérez J.F., Gasa J., Castrillo C. and Guada J.A., 1995. Effect of reproductive state and concentrate supplementation on liquid and particulate turnover in the rumen of ewes given ammonia treated straw. In: *Australian Journal of Agricultural Research,* 46, p. 1579-1586.

SAS, 2000. *User´s guide: Statistics*, Version 8 Edition. SAS Inst., Inc., Cary, NC.

Sylvester J.T., Karnati S.K.R., Yu Z.T., Morrison M. and Firkins, J.L., 2004. Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. In: *Journal of Nutrition,* 134, p. 3378-3384.

Valiente O.L., Delgado P., de Vega A. and Guada J.A., 2003. Validation of the n-alkane technique to estimate intake, digestibility, and diet composition in sheep consuming mixed grain: roughage diets. In: *Australian Journal of Agricultural Research,* 54, p. 693-702.

Van Kessel J.S. and Russell J.B., 1996. The effect of amino nitrogen on energetics of ruminal bacteria and its impact on energy spilling. In: *Journal of Dairy Science,* 79, p. 1237-1243.

Van Soest P.J., Robertson J.B. and Lewis B.A., 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. In: *Journal of Dairy Science,* 74, p. 3583-3597.