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Supplementation of cereal straws with different protein feeds: *In vitro* studies

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SUMMARY - Samples of the diets tested in 3 growth trials with lambs which were carried out at the Dipartimento di Scienze Zootecniche, our partner in Italy, were submitted to *in vitro* ruminal degradation in a semi-continuous fermentation system (RUSITEC). The aim of the study was the comparison between different protein feeds included in complete balanced diets, based on wheat straw, for growing lambs. The studied protein feeds were: soya bean meal (SBM), meat meal (MM), sunflower cake (SFC), coconut cake (CNC), brewers yeast (BY), fish meal (FM), blood meal (BM) and an industrial product with its protected protein (PP) from microbial attack, introduced at increasing levels of replacement of crude protein. The results clearly indicate that the diet with SBM was the most degradable. The diets with CNC and BM gave the highest and the lowest C_2/C_3 ratio, respectively. The diet with MM permitted the highest microbial protein yield (MPY). In the diets with the protected protein, the nitrogen degradation was only affected by the level of replacement and of crude protein and the best C_2/C_3 ratios were those of 30% protected protein and 18% crude protein.

Key words: Lambs feeding, protein feeds, *in vitro* fermentation.

RESUME - *"Supplémentation des pailles de céréales avec différents aliments protéiques : Etudes* in vitro*". Des échantillons de diètes qui ont été étudiées par des essais de croissance d'agneaux dans le Dipartimento di Scienze Zootecniche, notre partenaire en Italie, ont été soumis à dégradation ruminale* in vitro *dans un système semi-continu de fermentation (RUSITEC). Le but de l'étude a été la comparaison entre différents aliments protéiques qui étaient inclus dans des diètes complètes, basées sur la paille de blé et destinées à des agneaux en croissance. Les aliments protéiques étudiés étaient : farine de soja (SBM), farine de viande (MM), tourteau de tournesol (SFC), tourteau de coprah (CNC), levure de brasserie (BY), farine de poisson (FM), farine de sang (BM) et un produit industriel avec sa protéine protégée (PP) contre l'attaque microbienne, inclus à des niveaux croissants de remplacement de la protéine brute. Les résultats indiquent que la diète avec SBM était la plus dégradable. Les diètes avec CNC et BM ont donné le rapport C_2/C_3 le plus haut et le plus bas, respectivement. La diète avec MM a permis la production de protéine microbienne la plus haute. En ce qui concerne les diètes avec la protéine protégée, la dégradation de l'azote seulement a été influencée soit par le niveau de remplacement, soit par le niveau de protéine brute et les meilleurs rapports C_2/C_3 ont été ceux à 30% de protéine protégée et à 18% de protéine brute.*

Mots-clés : *Alimentation des agneaux, aliments protéiques, fermentation* in vitro.

Introduction

The need of diets containing high quality protein sources, slowly degraded in the rumen is a reality for high producing ruminants. Protein supply to the intestinal tract for absorption is derived from microbial protein synthesized in the rumen, dietary protein that escapes ruminal degradation and endogenous protein (Garret *et al.*, 1987; Poncet *et al.*, 1995). However, microbial protein accounts for an important level of amino acids entering the duodenum, but the depressing effect of low degradable protein sources on microbial protein flow and on Rumania degradation rates many times observed, impairs the benefits for the host animal.

Two studies were performed to compare either different protein sources included into isonitrogenous complete diets based on wheat straw and maize meal, or a combination of protein inclusion and protection levels in concentrate based diets for growing lambs and their balance with microbial products in a semi-continuous culture system. These *in vitro* studies were complementing the production trials made with growing lambs at University of Florence, Italy, using the same diets.

Material and methods

In the first phase of this project (1994-1995) seven diets containing ≈ 18-20% crude protein (CP) based on wheat straw (40%) and maize meal were formulated with the following protein sources: soya bean meal (SBM), blood meal (BM), brewers dried yeast (BY), fish meal (FM), meat meal (MM), sunflower meal (SFM) and coconut cake (CNC). These concentrates were studied taking the soya bean meal as a reference in two trials, being studied four protein sources in each one.

In a second phase (1996), sixteen diets were prepared using a commercial protected protein preparation claimed to be of low degradability at rumen level and providing four different levels of protein concentration in the diet (13.0, 15.5, 18.0 and 20.5% CP), without or with three levels of protection (15, 30 and 45%). The diets were prepared at the University of Florence and were mainly composed of straw, soya bean meal, maize, barley, the commercial protected protein product and a mineral vitamin mixture. Four trials were made, each one with four different diets.

A semi-continuous culture system, adapted from the Rumen Simulation Technique (RUSITEC) (Czerkawski and Breckenridge, 1977), was used to compare the rumen fermentation and the microbial protein synthesis yield of different protein sources in four trials each one with for different diets. This system was built in the laboratory of Estação Zootécnica Nacional (Portugal) and composed of four 1 l capacity vessels. On the first day each one was inoculated with strained rumen juice and solid rumen contents taken from 3 donor sheep fed with a standard diet of alfalfa pellets and straw. The samples of the diets (15 g of dry matter, DM) with a particle size of 3 mm, were introduced in nylon bags (160 x 80 mm) of 150 μm pore size. Each vessel contained 2 bags, each introduced on 2 consecutive days and removed 48 h after. The continuous infusion rate of a buffer medium was of 1 l d^{-1}. Measures were made during 5 days, after a 7 days adaptation period.

Both complete diets and bag residues were chemically analysed by the recommended methods of AOAC (1990) for dry matter, organic matter (OM), ash and nitrogen and for fibre composition by the method of Goering and van Soest (1970). Diets were also analysed for macrominerals after dry ashing by atomic absorption spectrophotometry.

Volatile fatty acids (VFA) in effluents were analysed by gas chromatography, using the procedure of Jouany (1981) and ammonia nitrogen (NH_3N) by a microdiffusion method (OJEC, 1971).

The difference between total nitrogen and ammonia nitrogen was used to evaluate microbial nitrogen production. The fermented organic matter (FOM) was calculated from the molar output of VFA by the stoichiometric equation of Demeyer and van Nevel (1975).

For the statistical treatment of the results, each one of the studied parameters was compared by means of variance analysis. When significant differences were obtained, multiple range testes (least significant difference, LSD) were made to detect probable differences between diets.

Results and discussion

Study of different protein sources on ruminal degradative and fermentative parameters in a semi-continuous fermentation system

Table 1 shows the chemical composition of the diets and it can be seen that the crude protein content (Total N x 6.25) varied between 20.8 and 18.0% DM for respectively both BY and FM and CNC. CNC presented the highest values of fibre content [50.2 and 31.3% DM for respectively neutral detergent fibre (NDF) and acid detergent fibre (ADF)]. The analysis data are slightly different from those produced by the Italian laboratory of Florence, probably because of sampling.

Tables 2 and 3 show the degradative and fermentative parameters observed in RUSITEC for the studied diets.

Table 1. Ingredient and chemical composition of tested diets in the first two experiments, as analysed at EZN

	1994				1995			
	SBM	MM	SFC	CNC	SBM	BY	FM	BM
Ingredients (%)								
Wheat straw	40	40	40	40	40	40	40	40
Maize meal	25	27	18	–	25	23	21	34
Soya bean meal	30	15	15	15	30	15	15	15
Meat meal	–	13	–	–	–	–	–	–
Sunflower cake	–	–	22	–	–	–	–	–
Coconut cake	–	–	–	40	–	–	–	–
Brewers dried yeast	–	–	–	–	–	17	–	–
Fish meal	–	–	–	–	–	–	9	–
Blood meal	–	–	–	–	–	–	–	6
Minerals & vitamins	5	5	5	5	5	5	5	5
Nutrients (% DM)								
Dry matter	87.2	85.9	86.8	86.9	89.8	90.0	90.2	90.1
Crude protein	19.4	19.6	19.4	18.0	19.5	20.8	20.8	19.4
Ash	9.5	9.9	7.7	8.9	11.8	11.7	11.0	10.2
NDF	32.9	42.6	39.1	50.2	34.2	42.6	35.4	39.2
ADF	20.8	25.0	25.8	31.3	16.7	20.7	19.5	18.1
ADL†	4.1	4.7	4.6	5.4	2.5	2.7	2.2	2.8
AIA††	2.2	2.7	1.8	2.3	2.1	2.3	2.6	1.8
DM *in vitro* digestibility	73.9	74.3	72.8	69.7	76.6	74.0	72.2	69.0
OM *in vitro* digestibility	72.8	73.1	71.7	67.4	76.4	71.7	70.4	68.0

†ADL: acid detergent lignin
††AIA: acid insoluble ash

In relation to the degradative parameters, it was observed that SBM presented values significantly higher ($P < 0.05$) than the other diets. Although not significantly different from BM and FM, BY showed the lowest level of fibre degradation, reflected in a significantly lower ($P < 0.05$) dry matter degradation. For results presented in Table 1 it can be said that with exception of fibre, the degradation percentages of the studied parameters, were significantly different ($P < 0.05$) among diets, presenting SBM the highest values.

Regarding the percentage of OM degradation, it was observed that it follows the same pattern of FOM estimated from VFA production with values varying between 88.0/4.89% and 82.9/4.46% respectively for SBM and BY.

For the molar percentage of VFA, BM showed to be the most fermentable, producing more propionate ($P < 0.05$) and BY showed to be the less fermentable with the highest ($P < 0.05$) level of acetate, which agrees with the values observed for the degradative parameters.

Microbial N, microbial protein synthesis yield and gas production were not significantly affected by the type of diet.

Table 2. Degradative and fermentative parameters observed in RUSITEC for the studied diets (1994 trial)

	SBM	MM	SFC	CNC
Degradation (48 h) (%)				
DM	83.80 ± 1.67^{a}	79.10 ± 1.31^{b}	80.70 ± 2.95^{b}	71.80 ± 4.39^{c}
OM	83.20 ± 1.78^{a}	78.20 ± 1.42^{b}	80.20 ± 3.12^{ab}	70.60 ± 4.60^{c}
N	91.80 ± 2.17^{a}	90.80 ± 1.69^{ab}	88.10 ± 3.38^{b}	84.30 ± 3.58^{c}
NDF	62.00 ± 5.65^{a}	62.10 ± 4.51^{a}	63.20 ± 7.43^{a}	56.10 ± 9.08^{a}
Total VFA (mM l^{-1})	40.80 ± 3.54^{a}	40.30 ± 1.63^{a}	38.30 ± 1.56^{a}	40.10 ± 4.00^{a}
Molar % VFA				
C_2	45.00 ± 0.69^{b}	47.00 ± 2.27^{b}	45.00 ± 3.60^{b}	54.00 ± 0.54^{a}
C_3	37.30 ± 1.22^{a}	35.50 ± 2.59^{a}	37.30 ± 3.95^{a}	30.90 ± 1.36^{b}
C_4	8.80 ± 0.88^{b}	9.80 ± 0.76^{a}	9.60 ± 0.59^{a}	8.80 ± 0.69^{b}
SC†	9.00 ± 0.28^{a}	7.70 ± 0.20^{b}	7.70 ± 0.17^{b}	6.30 ± 0.23^{c}
FOM (g d^{-1})††	4.03 ± 0.33^{a}	3.89 ± 0.31^{a}	3.71 ± 0.23^{a}	3.74 ± 0.49^{a}
Microbial N (mg d^{-1})†††	145.20 ±41.60^{b}	218.70 ±78.90^{a}	148.70 ±34.10^{b}	129.00 ±24.50^{b}
MPY (g mN kg^{-1} FOM)	36.60 ±12.90^{b}	56.30 ±19.30^{a}	39.90 ± 7.89^{b}	34.70 ± 5.31^{b}
Gas production (ml g^{-1} d^{-1})	198.00 ±41.70^{a}	186.00 ±21.80^{a}	191.80 ±19.00^{a}	171.50 ±19.70^{b}

†SC = $isoC_4$ + $isoC_5$ + C_5 + C_6
††FOM = (C_2/2 + C_3/2 + C_4 + C_5) x 162
†††Microbial N = Total N - NH_3N
^{a,b,c}Values in rows with different letters are significantly different at $P < 0.05$

Table 3. Degradative and fermentative parameters observed in RUSITEC for the studied diets (1995 trial)

	SBM	BM	BY	FM
Degradation (48 h) (%)				
DM	87.70 ± 0.99^{a}	84.90 ± 1.21^{bc}	83.30 ± 2.05^{c}	85.90 ± 2.15^{b}
OM	88.00 ± 0.87^{a}	84.40 ± 1.35^{b}	82.90 ± 2.09^{b}	85.50 ± 2.24^{ab}
N	94.80 ± 0.96^{a}	93.60 ± 1.24^{b}	92.70 ± 0.98^{b}	91.00 ± 1.70^{c}
NDF	74.20 ± 2.19^{a}	70.00 ± 2.89^{b}	68.40 ± 3.95^{b}	69.70 ± 4.75^{b}
ADF	68.50 ± 2.74^{a}	60.50 ± 3.86^{b}	60.20 ± 5.84^{b}	67.60 ± 5.10^{a}
Total VFA (mM l^{-1})	46.90 ± 2.93^{ab}	46.60 ± 2.93^{ab}	44.80 ± 2.58^{b}	49.20 ± 4.26^{a}
Molar % VFA				
C_2	55.30 ± 1.57^{c}	56.60 ± 0.59^{b}	58.00 ± 0.60^{a}	56.80 ± 1.62^{b}
C_3	17.10 ± 0.29^{b}	22.70 ± 1.11^{a}	17.80 ± 0.64^{b}	17.70 ± 1.23^{b}
C_4	19.40 ± 1.01^{a}	12.20 ± 0.79^{c}	16.70 ± 0.36^{b}	17.10 ± 1.24^{b}
SC†	5.41 ± 0.28^{a}	5.25 ± 0.20^{a}	4.75 ± 0.17^{b}	5.59 ± 0.23^{a}
FOM (g d^{-1})††	4.89 ± 0.39^{a}	4.48 ± 0.22^{bc}	4.46 ± 0.30^{c}	4.81 ± 0.48^{ab}
Microbial N (mg d^{-1})†††	154.40 ± 50.00^{a}	140.40 ± 52.00^{a}	124.90 ± 49.40^{a}	159.00 ±58.60^{a}
MPY (g mN kg^{-1} FOM)	31.90 ± 11.40^{a}	31.70 ± 12.30^{a}	27.90 ± 11.00^{a}	33.30 ±12.50^{a}
Gas production (ml g^{-1} d^{-1})	184.30 ± 21.50^{a}	194.40 ± 11.60^{a}	193.90 ± 6.70^{a}	188.70 ±17.30^{a}

†SC = $isoC_4$ + $isoC_5$ + C_5 + C_6
††FOM = (C_2/2 + C_3/2 + C_4 + C_5) x 162
†††Microbial N = Total N - NH_3N
^{a,b,c}Values in rows with different letters are significantly different at $P < 0.05$

From all the obtained results it can be concluded that SBM presented the best values in this group of diets, remaining BY in the opposite side.

For CNC the greater quantities of dietary protein that escaped ruminal degradation, were balanced by lower microbial protein synthesis. As a reflex of lower protein availability, microbial activity was affected with lower values of DM, OM and NDF degradations, being the extreme values observed between SBM/MM and CNC. Regarding VFA, although the total concentrations were not significantly different among diets, the molar concentrations of acetate and propionate were affected ($P < 0.05$) by dietary protein source, agreeing the values observed for CNC, with the degradative parameters observed.

For SFC there was some discrepancy between the loss of OM from the bags and the amount of FOM calculated from the production of VFA's. Gas production agrees with the observed degradation results, presenting CNC values significantly lower ($P < 0.05$) than the other diets.

The values observed for MM show the quality of this diet in relations to the other protein sources, mainly in what concerns microbial N and microbial protein synthesis yield. This protein source has also been considered the best for nitrogen and energy retention in production trials with sheep (Bruni, 1995; Antongiovanni *et al.*, 1996).

Taking SBM as a reference and comparing the two groups of diets (Tables 1 and 2) studied in 1994 and 1995 we can say that differences were observed between them, even for the reference feed (SBM).

This fact can be attributed to differences in the manufacture of the diets, to the higher protein content of the diets (≈ 20%), or, to the processing during the *in vitro* incubation, what is less probable, because during the production trials with lambs, the same tendency was observed (Antongiovanni *et al.*, 1996).

The effect of different protein sources and levels on ruminal degradative and fermentative parameters in a semi-continuous fermentation system

Table 4 shows the chemical composition of the diets studied in the third experiment and it can be seen that the actual crude protein contents (Total N x 6.25) were higher than the theoretically calculated ones and the differences expected between the diets (15.5/18.0%) were not observed for the groups of 15, 30 and 45% of protected protein (18.3/19.3, 19.5/19.3 and 18.2/19.8% respectively). Also it is interesting to mention the decrease of the starch content of the diets with the increase of protein level which may be due to the balance between soya bean meal and the maize + barley inclusion in the diets. However this tendency was not observed for the group of 30% of protein protection.

Table 5 shows the treatment effects on the different parameters studied, obtained by the variance analysis relatively to both factors protein protection and protein level.

Regarding DM and OM degradabilities significant differences were not observed neither due to the protection nor to the inclusion of protein, although the lowest values observed were, as expected, for the higher level of protein protection and the lower level of protein inclusion (68.7/68.2% and 67.5/67.6%, respectively). However significant differences ($P < 0.05$) were observed for those parameters due to the factor interactions.

Looking at nitrogen degradations significant differences ($P < 0.05$) were observed due either to the protection or to the inclusion of protein, but not as it would be expected, as the levels of 30 and 45% of the protein protection showed the highest values of nitrogen degradation (79.4 and 78.2% respectively). The effect of inclusion was observed and we can say that there was more protein that because was more available.

Relatively to the fibre fractions significant differences ($P < 0.05$) were observed for NDF degradation due to the protection of protein, decreasing those values with the level of protection. The same tendency was observed for ADF however it was not so evident. The percentage of protein inclusion increased significantly ($P < 0.01$) the NDF and the ADF degradations.

Table 4. Chemical composition of diets (% DM) of the 1996 experiment, as analysed at EZN

Diet	DM	CP	Ash	AIA	Soluble sugar	Starch	NDF	ADF	ADL	Ca	P
1	88.1	15.6	8.3	3.7	2.9	28.6	46.3	26.9	3.3	0.9	0.3
2	89.6	16.7	8.8	3.7	4.3	26.6	46.2	28.4	3.6	0.7	0.4
3	88.5	19.5	11.4	5.1	2.6	21.7	47.5	27.3	3.7	1.0	0.4
4	87.5	22.8	11.4	5.1	3.3	16.6	48.1	28.5	3.7	1.0	0.5
5	88.7	15.6	9.7	4.5	3.8	32.8	43.8	24.0	3.2	0.9	0.4
6	88.1	18.3	11.3	5.1	3.2	28.4	43.8	25.4	3.2	1.1	0.4
7	88.4	19.6	12.6	5.7	4.7	22.0	40.9	24.3	3.2	1.2	0.5
8	88.2	21.8	10.5	5.5	3.8	17.6	49.2	27.5	3.7	1.0	0.4
9	88.9	14.5	10.3	4.9	2.9	31.2	43.9	26.8	4.0	1.0	0.4
10	89.6	19.5	12.6	5.9	4.4	21.2	44.3	27.6	4.2	1.2	0.5
11	89.3	19.3	9.9	4.2	4.3	22.1	44.2	24.8	3.3	0.8	0.4
12	89.1	21.1	10.1	4.7	3.1	24.8	46.5	30.6	4.0	0.9	0.5
13	88.4	15.7	11.3	5.1	2.9	29.4	44.7	26.9	3.7	1.3	0.4
14	88.5	18.2	9.6	4.3	4.4	26.1	41.2	27.4	3.6	0.9	0.4
15	89.1	19.8	12.1	5.3	3.5	24.3	45.5	31.5	4.0	1.2	0.5
16	88.0	22.8	12.0	5.3	4.0	18.0	43.1	26.8	3.1	1.3	0.4

Total VFA production was impaired and was significantly lower ($P < 0.01$) for the two highest levels of protein protection, but it was not affected by the level of protein inclusion. Regarding the molar percentage of VFA, significant differences ($P < 0.01$) were observed due to the effect of protection and inclusion of protein, but not for acetate and butyrate productions, which were not affected by the level of protein protection. The level of 20.5% protein inclusion has shown as the "most fermentable", producing more propionate and less acetate. Significant interactions ($P < 0.01$) were observed between treatments for total VFA production and molar percentage of VFA.

Fermentable organic matter was only significantly affected ($P < 0.01$) by the percentage of protein protection, decreasing for the two highest levels of protection and following the same pattern of OM degradation. Neither microbial N nor microbial protein synthesis yield were affected by the treatments. Here we must consider the low precision of the method used for calculating the microbial nitrogen (Total N - ammonia N) which can also be seen for the values of microbial protein synthesis yield. Also, high daily variations were seen for these parameters, which were responsible for very high confidence levels observed. Gas production was affected ($P < 0.01$) by the protein protection and protein inclusion and also by the treatment interactions. It is interesting to see that gas production was related to the soluble sugar content but not with the starch level of the diets.

As expected, pH of effluents were lower than those of fermenters, due to the microbial VFA production, following the latter the same pattern of total VFA production. Only the pH of fermenters was significantly affected ($P < 0.01$) by the level of protein protection. It can be concluded that the best values were observed for the two higher levels of protein not protected, i.e. completely available.

When looking into the protection of protein it seems from this experiment that for the lowest protection level (15%) the diet must have at least 18% of crude protein. If a higher protection level is pretended (30 or 45%) and taking into consideration the degradative parameters, then it is better to use a 20.5% level of protein inclusion. But if one wants also to optimize the microbial protein production, which is also available for intestinal absorption, then, and for the 30% level of protection, the inclusion rate must be at least 15.5% of protein when the degradation parameters are less affected. For the 45% protection level the inclusion of protein must be 20.5% when there is high microbial protein production and high rates of degradation but affecting negatively the fibre degradation rate.

Table 5. Treatment effects on the different degradative and fermentative parameters studied in RUSITEC (diets of the 1996 growth trial)

	Level of protected protein (% of CP)				Crude protein level (% DM)				Significance		
	0	15	30	45	13.0	15.5	18.0	20.5	PP	CP	PP x CP
Degradation (48 h) (%)											
DM	69.53	68.89	69.15	68.65	67.54	69.72	69.31	70.67	NS†	NS	*
OM	70.16	70.29	68.63	68.17	67.61	69.72	69.27	70.66	NS	NS	*
N	75.39^{ab}	73.78^{a}	79.38^{b}	78.24^{b}	75.54^{a}	74.38^{a}	76.70^{ab}	80.19^{b}	*	*	NS
NDF	52.24^{b}	49.78^{ab}	46.92^{a}	45.87^{a}	44.05^{a}	47.05^{ab}	49.92^{bc}	53.78^{c}	*	**	**
ADF	42.25	38.10	37.49	38.54	32.10^{a}	40.10^{b}	39.33^{b}	44.85^{c}	NS	**	**
Total VFA (mM l^{-1})	40.57^{b}	44.86^{c}	36.81^{a}	36.47^{a}	39.67	40.74	39.38	38.92	**	NS	**
Molar % VFA											
C_2	51.24	52.49	50.87	50.99	51.05^{a}	51.00^{a}	50.36^{a}	53.14^{b}	NS	**	**
C_3	26.40^{a}	25.95^{a}	30.45^{b}	29.83^{b}	28.01^{b}	28.84^{bc}	29.45^{c}	26.32^{a}	**	**	**
C_4	14.50^{b}	13.74^{b}	11.37^{a}	11.79^{a}	14.29^{b}	12.78^{a}	12.01^{a}	12.32^{a}	**	**	**
SC††	7.57	7.81	7.12	7.39	6.57^{a}	7.37^{b}	8.04^{c}	7.91^{bc}	NS	**	**
FOM (g d^{-1})†††	3.79^{b}	3.76^{b}	3.32^{a}	3.24^{a}	3.62	3.63	3.40	3.46	**	NS	NS
Microbial N (mg d^{-1})††††	173.32	154.35	168.02	159.50	159.28	160.13	171.69	164.10	NS	NS	NS
MPY (g mN kg^{-1} FOM)	45.90	41.33	51.68	49.18	44.45	44.49	51.03	48.11	NS	NS	NS
Gas (ml g^{-1} d^{-1})	156.49^{a}	197.66^{b}	159.69^{a}	161.71^{a}	198.13^{b}	148.62^{a}	135.43^{a}	186.71^{b}	**	**	**
pH fermenters	6.86^{ab}	6.82^{a}	6.94^{c}	6.92^{bc}	6.86	6.86	6.89	6.93	**	NS	NS
pH effluents	6.83	6.81	6.86	6.87	6.85	6.83	6.84	6.86	NS	NS	**

†NS: non significant; *P < 0.05; **P < 0.01
††SC = $isoC_4$ + $isoC_5$ + C_5 + C_6
†††FOM = (C_2/2 + C_3/2 + C_4 + C_5) x 162
††††Microbial N = Total N - NH_3N
[a,b,c]Values in rows with different letters are significantly different at P < 0.05

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