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Moreira O.M.S.C., Ribeiro J.M.C.R.

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Utilization of different concentrates by growing lambs. Ruminal degradation

O.M.S.C. Moreira and J.M.C.R. Ribeiro
Estação Zootécnica Nacional (INIA)
2000 Vale de Santarém, Portugal

SUMMARY - Sixteen diets based on straw, soya bean meal, maize and barley, were prepared with a commercial protected protein product, claimed to be of low degradability at rumen level and providing four levels of protein concentration in the diet (13.0, 15.5, 18.0 and 20.5% CP) without or with three levels of protein protection (15, 30 and 45%). A semi-continuous culture system adapted from the rumen simulation technique (RUSITEC) was used to compare the effects of the different levels of protein protection and inclusion on ruminal degradative and fermentative parameters. 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 absortion, 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.

Key words: Protein, supplementation, bypass, ruminal degradation, *in vitro*.

RESUME - *"Utilisation de différents concentrés par des agneaux en croissance. Dégradation ruminale". Seize diètes à base de paille, farine de soja, maïs et orge, étaient preparées avec une mélange commerciale de protéine protegée, peu dégradable au niveau du rumen, fournissant quatre niveaux d'inclusion de matières azotées totaux (13,0, 15,5, 18,0 et 20,5% MAT), sans ou avec trois niveaux de protéction (15, 30 et 45%). Un système semi-continu de type RUSITEC était utilisé pour comparer l'effet des niveaux soit d'inclusion, soit de protéction de protéine, sur des paramètres de dégradation et de fermentation au niveau du rumen. En ce qui concèrne le niveau de protection on a constaté que pour le plus bas (15%), la diète devra avoir ou moins 18% de protéine brute, mais si un niveau supérieur est prétendu, ayant en considération les paramètres de dégradation, alors une inclusion de proteíne est conseillée. Rélativement à l´optimization de la production de proteíne microbienne, elle aussi disponible pour l'absorption intéstinale, pour le niveau de 30% de protection, l'inclusion sera du moins 15,5%. Pour une protection de 45%, le niveau d'inclusion sera de 20,5%, valeur pour laquelle on a observé une meilleur production de protéine microbienne, étant cependant les taux de dégradation de la fibre déjà négativement affectés.*

Mots-clés : *Protéine, supplementation, protection, dégradation ruminale,* 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 effects of low degradable protein sources on microbial protein flow and on ruminal degradation rates many times observed, imparts the benefits for the host animal.

The objective of this study was to compare a combination of protein inclusion and protection levels in concentrate based diets for growing lambs and its balance with microbial products in a semi continuous culture system.

Materials and methods

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.

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 four different diets. This system was built in our laboratory 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 concentrates (15 g 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 buffer medium was of 1 l/day. 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, ash and nitrogen and for fibre composition by the method of Goering and van Soest (1970). Diets were also analysed for the 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 microdifusion 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, two factors were considered: the percentage of protein protection and the percentage of protein inclusion. Each one of the studied parameters was compared by means of variance analysis where the reported factors were considered as well as their possible interactions. When significant differences were obtained multiple range tests (LSD) were made to detect probable differences between the levels of two factors.

Results and discussion

Table 1 shows the chemical composition of the diets and it can be seen that the effective crude protein content (Total N x 6.25) varied between 15.6 and 22.8%. All the observed values were higher than the theoretically calculated values and the differences expected between the diets (15.5/18.0%) were not observed for the groups of 15, 30 and 45% of protein protected (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 2 shows the treatment effects on the different parameters studied, obtained by the variance analysis relatively to the 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.

Table 1. Chemical composition of the different concentrates

% Protein protection	% Protein inclusion	DM (%)	OM	CP	ASH	Si	Sol. Sugar	Starch (% MS)	NDF	ADF	ADL	Ca	P
0	13.0	88.1	91.71	15.6	8.29	3.70	2.88	28.58	46.30	26.90	3.32	0.90	0.32
	15.5	89.6	91.25	16.7	8.75	3.70	4.34	26.56	46.20	28.40	3.55	0.73	0.43
	18.0	88.5	88.62	19.5	11.38	5.10	2.63	21.68	47.50	27.30	3.72	1.03	0.44
	20.5	87.5	88.59	22.8	11.41	5.10	3.27	16.57	48.10	28.50	3.68	1.01	0.54
15	13.0	88.7	90.35	15.6	9.65	4.50	3.83	32.83	43.80	24.00	3.17	0.89	0.42
	15.5	88.1	88.73	18.3	11.27	5.10	3.15	28.43	43.80	25.40	3.23	1.07	0.42
	18.0	88.4	87.42	19.6	12.58	5.70	4.67	21.99	40.90	24.30	3.19	1.20	0.54
	20.5	88.2	88.46	21.8	10.54	5.50	3.77	17.55	49.20	27.50	3.73	1.00	0.44
30	13.0	88.9	89.72	14.5	10.28	4.90	2.92	31.17	43.90	26.80	4.01	0.99	0.37
	15.5	89.6	87.44	19.5	12.56	5.90	4.37	21.17	44.30	27.60	4.18	1.16	0.52
	18.0	89.3	90.08	19.3	9.92	4.20	4.25	22.14	44.20	24.80	3.28	0.82	0.39
	20.5	89.1	89.85	21.1	10.15	4.70	3.06	24.84	46.50	30.60	3.95	0.94	0.46
45	13.0	88.4	88.69	15.7	11.31	5.10	2.89	29.43	44.70	26.90	3.69	1.25	0.43
	15.5	88.5	90.37	18.2	9.63	4.30	4.42	26.14	41.20	27.40	3.64	0.87	0.37
	18.0	89.1	87.89	19.8	12.11	5.30	3.46	24.34	45.50	31.50	4.01	1.15	0.49
	20.5	88.0	87.97	22.8	12.03	5.30	3.97	17.99	43.10	26.80	3.14	1.31	0.40

Table 2. Treatment effects on the different degradative and fermentative parameters studied in Rusitec

	% Protein protection				% Protein inclusion				S		
	0 (n=32)	15 (n=32)	30 (n=32)	45 (n=32)	13.0 (n=32)	15.5 (n=32)	18 (n=32)	20.5 (n=32)	Prot.	Incl.	Prot. X Incl.
Degradation (48 h)											
% DM	69.53	69.89	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	**	**
T VFA (mM/1000 ml)	40.57^{b}	44.86^{c}	36.81^{a}	36.47^{a}	39.67	40.74	39.38	38.92	**	NS	**
Molar % VFA											
C2	51.24	52.49	50.87	50.99	51.05^{a}	51.00^{a}	50.36^{a}	53.14^{b}	NS	**	**
C3	26.40^{a}	25.95^{a}	30.45^{b}	29.83^{b}	28.01^{b}	28.84^{bc}	29.45^{c}	26.32^{a}	**	**	**
C4	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/day)††	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/day)†††	173.32	154.35	168.02	159.50	159.28	160.13	171.69	164.10	NS	NS	NS
MPSY (gmN/KgFOM)	45.90	41.33	51.68	49.18	44.45	44.49	51.03	48.11	NS	NS	NS
GAS (ml/g/day)	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	**

†SC: isoC4 + isoC5 + C5 + C6
††FOM: (C2/2 + C3/2 + C4 + C5) x 162
†††Microbial N: total N - NH_3 N
S: Significance for an error level of P<0.05 (*) or P<0.01 (**)
a,b,c: For each treatment values in rows with different letters are significantly different at the indicated significance level; NS: non significant

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).

With regard 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.

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. 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 observed 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.

Conclusions

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 the degradative parameters into consideration, 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.

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