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Original article

Effect of type of lucerne hay on caecal fermentation and nitrogen contribution through caecotrophy in rabbits †

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Summary — Seventy-five New Zealand White x Californian rabbits were used to study the influence of the chemical composition of lucerne hay on caecal and caecotrophy characteristics. Five lucerne hays varying in chemical composition were ground and formed into pellets. These were the sole form of nutrition during the experiment. The type of lucerne hay did not affect caecal volatile fatty acid concentration, pattern of fermentation or pH. However, the caecal ammonia concentration decreased linearly (by 30% between extreme diets, $P = 0.002$) when dietary fibre proportion increased. The weight of caecum and caecal contents increased linearly (by 12%, $P = 0.010$, and 35%, $P < 0.001$, respectively, between extreme diets) with dietary fibre proportion. Soft faeces excretion and contribution of soft faeces to dry matter intake were not influenced by the type of lucerne hay. The proportion of caecal content that appeared daily as soft faeces and the total and microbial nitrogen concentrations in soft faeces were higher (42, 14 and 39%, respectively) for the lucerne hay with the lowest dietary fibre proportion than for the average of the other hays.

rabbit / lucerne hay / caecum

Résumé — L'effet du type de foin de luzerne sur la digestion chez les lapins. *Dans cette expérience 75 lapins issus du croisement Néo-Zélandais Blanc x Californien ont été utilisés pour l'étude de l'influence de la composition chimique du foin de luzerne sur les caractéristiques cæcales et la cæcotrophie. Cinq foins de différentes compositions chimiques ont été broyés et fournis comme aliments uniques durant l'essai. Le type du foin de luzerne n'a affecté ni la concentration des AGV cæcaux, ni le profil de fermentation, ni le pH, alors que la concentration de l'ammoniaque cæcal diminue linéairement quand la proportion de fibres dans la ration augmente (de 30% entre les rations extrêmes, P = 0,002). Le poids du cæcum et les contenus cæcaux augmentent linéairement (de 12%, P = 0,010 et de 35%, P < 0,001, respectivement entre les rations extrêmes) avec le taux fibreux dans la ration. L'excrétion*

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des fèces molles et la contribution de ces dernières dans l'ingestion de la MS n'ont pas été affectées par le type du foin de luzerne. Le recyclage quotidien de contenu cæcal à travers les fèces molles et la concentration de l'azote total et microbien dans les fèces molles, pour le foin de luzerne ayant une faible proportion en fibres alimentaires, étaient plus élevés (42, 14 et 39%, respectivement) en comparaison avec la moyenne des autres foins.

lapins / foin de luzerne / cæcum

INTRODUCTION

Lucerne hay is a major ingredient of rabbit diets in Spain, accounting for approximately a third of commercial feeds. Lucerne hay is preferred to other sources of fibre because of its high palatability and estimable amino acid supply. Substitution of lucerne hay with highly lignified sources of fibre (as grape marc) led to a lower caecal volatile fatty acids (VFA) concentration (which promoted *in vitro Escherichia coli* proliferation; Prohaszka, 1980) and decreased nutrient digestibility (Motta, 1990; Fraga *et al,* 1991), whereas substitution with poorly lignified sources of fibre (as beet or citrus pulps) elicited an accumulation of digesta in the caecum (Motta, 1990; Fraga *et al,* 1991), which impaired digestible energy efficiency and decreased intake and productivity (García *et al,* 1993).

The chemical composition of lucerne hay is variable depending on the variety, maturity, climatology and drying conditions. These factors greatly affect the concentration of cell-wall carbohydrates and the protein contents. The coefficients of variation for crude protein and neutral detergent fibre (NDF) contents from 56 commercial samples were 9.0 and 14.0% (C Alvarez, 1993; unpublished results). Consequently, significant changes in hindgut digestion might also occur.

The objective of this work was to study the effect of type of lucerne hay on the pattern of caecal fermentation and its microbial contribution to rabbit requirements through caecotrophy. Lucerne hays used in this study were given as the sole feed, to avoid interactions with fibre content from other dietary ingredients.

MATERIALS AND METHODS

Diets

Five lucerne hays were selected out of 10 in order to obtain the greatest variability in fibre and protein composition, and were named A, B, C, D and E in increasing NDF order. The lucerne hays were ground, passed through a 4 mm screen, and formed into pellets (15 mm long x 3 mm diameter). The chemical analyses of these materials are shown in table I.

Digesta sampling trial

A group of 45 male and female New Zealand White x Californian rabbits (9 per diet), weighing from 1.5 to 1.6 kg, were randomly allotted to the 5 diets. Animals were given *ad libitum* access to the lucerne hay pellets, which were the sole form of nutrition throughout the experiment. After a 21 d adaptation period, the animals were slaughtered by cervical dislocation 1 h before dark (at 18.30 h), to avoid the caecotrophy period. They weighed 2.0 ± 0.1 (SD) kg on average. The gastrointestinal tract was removed and weighed. The stomach and caecum were weighed separately with and without their contents. The caecal content was removed, its pH measured, and it was divided into 2 samples. One was used to determine dry matter (DM), nitrogen (N), NDF and acid detergent lignin (ADL) contents. The other sample was centrifuged at 15 000 rpm at 0°C for 10 min. The supernatant fluid was used

Table I. Chemical composition of lucerne hays (% DM).

Item	*Lucerne hays*				
	A	*B*	*C*	*D*	*E*
DM	90.2	91.9	91.8	92.3	93.7
Ash	11.6	11.4	11.2	10.2	10.5
CF	24.8	29.9	28.7	30.9	37.2
NDF	38.7	47.8	48.9	49.0	55.0
ADF	29.5	35.5	35.8	36.7	40.2
ADL	6.0	8.0	7.9	8.0	9.0
UA	7.4	7.3	7.3	7.5	7.9
N	3.5	3.5	3.3	2.8	2.7
NDIN	0.5	0.8	0.7	0.5	0.7

DM: dry matter; CF: crude fibre; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; UA: uronic acids; N: nitrogen; NDIN: neutral detergent insoluble nitrogen.

to determine ammonia concentration and total concentration and molar proportion of VFA. A solution of 5% orthophosphoric acid (v/v) plus 1% mercury chloride (w/v) was added (0.1 ml ml^{-1}) to the samples for VFA determination. Samples for ammonia determination were acidified with a 0.2 M solution hydrochloric acid (1 ml ml^{-1}).

Caecotrophy trial

A second group of 30 male and female New Zealand White x Californian rabbits (6 animals per diet) weighing 1.75 ± 0.03 (SD) kg, were randomly allotted to the 5 diets. Animals were given *ad libitum* access to the lucerne hay pellets, which were the sole form of nutrition throughout the experiment. Following a 10 d adaptation period (2.0 kg of body weight), a wooden collar (25 cm diameter) was put on each animal to prevent caecotrophy. The collar was put on half an hour after light was switched on (at 8.00 h), and was removed 24 h later. The soft faeces collected were stored at –20°C. Feed intake and animal weight were recorded for 3 d before the collar was placed. Soft faeces were analysed for dry matter (DM), NDF, nitrogen (N), neutral detergent insoluble nitrogen (NDIN) and microbial nitrogen concentration.

Housing

The animals were housed in individual wire cages (245 x 610 x 315 mm high) in the digesta sampling trial. Metabolism wire cages (405 x 510 x 320 mm high), which allowed separation of faeces and urine, were used in the caecotrophy trial. The rabbits were kept in a closed building with partial environmental control, under a 12–12 h light–dark schedule. The light was switched on at 7.30 h. Temperature during the experimental period ranged from 14 to 22°C.

Analytical methods

Chemical analysis were performed using the method of Robertson and Van Soest (1981) for NDF, Goering and Van Soest (1970) for acid detergent fibre (ADF), ADL and neutral detergent insoluble nitrogen (NDIN), and AOAC (1984) for DM, ash, N and crude fibre (CF). Uronic acids were determined by the *m*-phenyphenol method (Blumenkrantz and Asboe-Hansen, 1973). Caecal ammonia was analyzed using the autoevaluation distillation unit Kilab nitrolab-auto. Samples were distilled with a solution of sodium tetraborate (2.5%), collected on boric acid solution (1%) and valorated with hydrochloric acid (0.05 M) and a

colour indicator. Caecal VFA concentration was determined in a Hewlett-Packard (5710 A) gas chromatograph, with a flame ionization detector, a Hewlett-Packard (3390 A) recorder integrator and a steel column using free fatty acids and phenols (FFAP) 10% H_3PO_4, 1% acid-washed chromosorb W, 100-120 mesh. The carrier gas was nitrogen with a flow rate of 30 ml min^{-1}; hydrogen and air flows to the detector were 30 and 200 ml min–1. Injection and detector temperatures were 250°C. The oven temperature was increased during the analysis from 110 to 160°C at a rate of 80°C min^{-1}.

The microbial nitrogen content in soft faeces was calculated from the purine nitrogen concentration. The samples of soft faeces were freeze-dried and their purine content was estimated using the total purine analysis method of Zinn and Owens (1980) as modified by Ushida *et al* (1985). A purine/bacterial nitrogen ratio of 0.89 mg RNA mg^{-1} of total nitrogen was previously determined on a bacterial preparation isolated from caecal contents of 20 does. These animals were fed a commercial diet composed of barley, sunflower meal, soyabean meal, corn gluten feed and lucerne hay as the main source of fibre. Bacteria of the caecal contents were isolated on the basis of the method proposed by Merry and McAllan (1983).

Caecal contents were suspended in saline (100 ml 200 g^{-1}) homogenized, stomached for 7 min and filtered through 46 μm nylon tissue. The filtrate contained both fluid and particle associated bacteria that were isolated together by differential centrifugation (Legay-Carmier and Bauchart, 1989). The mixture of bacteria cells was freeze-dried and subsequently analysed for total and purine nitrogen using the same analytical procedure described above for soft faeces.

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (1985). Data were blocked by sex. Data from digestive measurements trial were corrected using average daily gain and slaughter weight as covariates, whereas data from caecotrophy trial were corrected using the weight of the animal the day of soft faeces collection and the average daily intake 3 d before caecotrophy was avoided as covariates. Linear and quadratic effects of dietary NDF content were tested.

RESULTS

Digesta sampling trial

The weight of complete digestive tract, stomach, caecum and caecal contents (expressed as percentage of body weight) increased linearly by 0.268 ± 0.046 (P = 0.001), 0.009 ± 0.003 (P = 0.019), 0.011 ± 0.004 (P = 0.01) and 0.111 ± 0.024 (P = 0.001), respectively per each 1% increment of dietary NDF content on DM basis (table II), whereas feed intake and weight of stomach contents were not affected by diet. The differences between values observed for lucerne hays A and E were 25, 13, 12 and 35%, respectively.

NDF and ADL concentrations of caecal contents increased linearly (by 18 and 22%, from diet A to E, respectively) with dietary NDF proportion. The coefficients of regression were 0.421 ± 0.070 (P = 0.001) and 0.113 ± 0.030 (P = 0.001), respectively. Caecal DM content was not affected by the type of diet.

Nitrogen and ammonia concentration of the caecal contents decreased linearly with dietary NDF proportion (by 14 and 30%, from diet A to E, respectively). The coefficients of regression were –0.049 (P = 0.001) and –0.347 (P = 0.002), respectively.

The type of diet did not affect the pH, total VFA concentration or the pattern of fermentation (79.3, 6.6 and 14.1% as average for molar proportions of acetic, propionic and butyric acid, respectively).

Caecotrophy trial

The type of diet did not influence the daily feed intake, daily soft faeces excretion, or DM and NDIN content of soft faeces, which averaged 126.8 g, 20.8 g, 27.9% and 0.47%, respectively (table III). However, the

Table II. Effect of type of lucerne hays on caecal digestion traits (least square means).

Item	*Lucerne hays*					*SE* [a]	*L* [b]	*Q* [c]
	A	*B*	*C*	*D*	*E*			
DM intake (g d^{-1})	124	137	125	117	131	4.9	NS	NS
Digestive tract (% BW)	20.45	24.31	23.39	22.07	25.51	0.45	0.001	NS
Stomach								
Organ wt (% BW)	1.46	1.63	1.52	1.53	1.65	0.04	0.019	NS
Content wt (% BW)	4.74	5.16	4.94	4.70	5.34	0.22	NS	NS
Caecum								
Organ wt (% BW)	1.46	1.61	1.54	1.62	1.64	0.04	0.010	NS
Content wt (% BW)	5.17	6.52	6.47	5.85	7.16	0.25	0.001	NS
Caecal chemical composition								
DM (%)	19.6	19.4	19.4	20.3	18.8	0.27	NS	NS
NDF (% DM)	36.1	39.6	39.5	41.7	42.7	0.74	0.001	NS
ADL (% DM)	8.19	9.58	9.31	9.66	10.0	0.33	0.001	NS
N (% DM)	5.37	4.93	4.95	4.68	4.61	0.08	0.001	NS
NH_3-N (mmol l^{-1})	15.8	15.9	13.1	11.4	11.0	1.11	0.002	NS
pH	5.69	5.83	5.82	5.70	5.76	0.04	NS	NS
Total VFA (mmol l^{-1})	88.5	80.2	84.4	82.9	87.3	3.14	NS	NS
Acetic acid (%)	78.8	78.8	80.1	79.4	79.6	0.45	NS	NS
Propionic acid (%)	6.63	7.10	6.44	6.36	6.46	0.20	NS	NS
Butyric acid (%)	14.6	14.0	14.0	14.2	13.9	0.38	NS	NS

[a] SE: Mean standard error ($n = 9$). [b] L: Significance of linear effect of dietary NDF. [c] Q: Significance of quadratic effect of dietary NDF. NS: $P > 0.05$.

Table III. Chemical composition and excretion of soft faeces (least square means).

Item	*Lucerne hays*					*SE* [a]	*L* [b]	*Q* [c]
	A	*B*	*C*	*D*	*E*			
DM intake[d] (g d^{-1})	120	140	126	114	133	4.8	NS	NS
Soft faeces excretion								
g DM d^{-1}	22.7	20.5	18.8	20.2	21.6	2.53	NS	NS
g DM 100^{-1} g^{-1} BW d^{-1}	1.19	1.07	0.90	1.06	1.13	0.13	NS	NS
Soft faeces chemical composition								
DM (%)	27.1	28.1	28.5	27.2	28.6	0.80	NS	NS
NDF (% DM)	37.5	41.8	41.5	42.7	44.1	0.89	0.001	NS
N (% DM)	5.20	4.56	4.81	4.29	4.52	0.11	0.001	NS
NDIN (% DM)	0.49	0.40	0.49	0.49	0.50	0.03	NS	NS
Microbial nitrogen[e] (% DM)	2.10	1.65	1.60	1.32	1.47	0.08	0.001	0.041
Contribution to total DM intake[f] (%)	16.0	15.2	13.8	13.8	16.7	1.79	NS	NS
Contribution to N intake[g] (%)	21.6	18.7	18.4	19.3	24.3	2.07	NS	0.025

[a] SE: mean standard error ($n = 6$). [b] L: significance of linear effect of dietary NDF. [c] Q: significance of quadratic effect of dietary NDF. [d] Average DM intake 3 d before collection. [e] Microbial nitrogen calculated from purine/nitrogen ratio (0.89 mg yeast RNA mg^{-1} total nitrogen of bacterial preparation) ($n = 6$). [f] As (soft faeces excretion (g DM d^{-1}) x 100/(feed intake (g DM d^{-1}) + soft faeces excretion (g DM d^{-1})). [g] As (N excreted in soft faeces (g d^{-1}) x 100/(N ingested in feed (g d^{-1}) + N excreted in soft faeces (g d^{-1})). NS: $P > 0.05$.

dietary NDF proportion linearly affected the soft faeces concentrations of NDF and total nitrogen, which increased and decreased by 0.413 ± 0.077 (P = 0.001) and 0.047 ± 0.015 (P = 0.001) for each 1% increment of dietary NDF content on a DM basis, respectively.

The calculated proportions of microbial nitrogen (% DM basis) decreased linearly (P = 0.001)with dietary NDF concentration (by 0.055 ± 0.012 for each percentage unit of increase of NDF).

The contribution of soft faeces to nitrogen intake was influenced quadratically (P = 0.025) by dietary NDF proportion, showing a minimum value (13.8%) for the lucerne hays C and D, whereas the contribution to DM intake was not affected by the type of lucerne hay (20.5% on average).

DISCUSSION

The caecum of rabbits fed lucerne hay as their sole feed was characterized by a relatively high concentration of VFA and a low caecal pH (84.8 mmol l^{-1} and 5.76 on average, respectively). Rabbits fed compound diets with a lower fibre proportion (Carabaño *et al,* 1988; Parigi-Bini *et al,* 1990; Fraga *et al,* 1991) showed lower concentrations of VFA and higher values of pH (43.5, 64.0 and 52.5 mmol l^{-1} and 5.86, 5.90 and 6.07 respectively). The values obtained in this study are in agreement with those reported by Gidenne (1986) in animals fed a diet containing 90% lucerne (97.4 mmol l^{-1} and 5.68). These results might be explained because of a higher availability of substrates for fermentation is found in lucerne hay than in less fibrous diets. In the same way, an increase of concentration of VFA (from 18.1 to 30.0 mmol l^{-1}) and a reduction of pH (from 6.18 to 6.02) when increasing the the digestible NDF proportion (from 3.5 to 6.5%), has been reported by Gidenne (1990), using lucerne hay as sole source of fibre. Accordingly, an increase in dietary lucerne hay proportion seems to lead to a higher caecal content acidity (lower pH and higher VFA concentrations), which helps to control the proliferation of *E coli* according to Prohaszka (1980). The lack of effect of type of diet on these traits in this study might be explained by the slight differences among diets in digestible NDF content (10.7 and 11.2% in diets A and E, respectively; García *et al,* 1995).

Caecal ammonia concentrations ranged from 11 to 16 mmol N-NH_3 l^{-1} in this study. An even higher value (23.6 mmol N-NH_3 l^{-1}) was observed in a diet containing 90% lucerne hay by Gidenne (1986). These results are higher than those obtained with balanced diets (around 4.4 mmol N-NH_3 l^{-1}; Carabaño *et al,* 1988; Parigi-Bini *et al,* 1990; Fraga *et al,* 1991) and might be explained by the low energy to protein ratio (which ranged from 46.8 to 61.8 kJ DE g^{-1} digestible crude protein in the diets of this study; García *et al,* 1995) with respect to rabbit growth requirements (98.3 kJ g^{-1}; de Blas *et al,* 1981). Recycling of urea from blood to caecum accounts for a high proportion of caecal ammonia (Forsythe and Parker, 1985a, b) and might be increased when the protein intake exceeds the requirements. An excess of dietary protein, leading to increases in caecal ammonia and pH, has been related to proliferation of *Clostridium spiroforme* (Haffar *et al,* 1988) and a higher incidence of diarrhoea (de Blas *et al,* 1981; Haffar *et al,* 1988). In the same way, Catala and Bonafous (1979), reducing the protein digestibility by ligature of the pancratic duct, observed an increase in microbial proliferation in the hindgut. Our results support the need to balance the dietary energy to protein ratio in order to keep the caecal ammonia concentration at minimal levels. The effect of type of diet on the caecal ammonia concentration observed in this study was related to digestibility variables and dietary chemical composition using regression procedures. The best correlations were obtained

with digestible ($r = 0.541$, $P < 0.001$) and soluble (total nitrogen – NDIN; $r = 0.510$, $P < 0.001$) nitrogen. An increase of caecal ammonia concentration (from 4.7 to 7.6 mmol $N\text{-}NH_3$ l^{-1}) has also been observed by Carabaño *et al* (1989) when substituting for lucerne hay, sunflower meal and barley straw. This increased crude protein digestibility from 0.55 to 0.71.

The weight of the stomach and caecal contents were similar to those obtained in a diet containing 90% lucerne hay (Gidenne, 1986), and higher than those reported for less fibrous diets (Carabaño *et al*, 1988; García *et al*, 1993). An increase of lucerne NDF content increased linearly the weight of caecal content which agrees with previous results (Gidenne, 1992).

The soft faeces excretion was not affected by the type of lucerne hay, the average was 20.8 g DM d^{-1}. The value is within the range reported in the literature using growing rabbits fed compound diets (Gidenne and Lebas, 1987; Carabaño *et al*, 1988; Motta, 1990).

Caecal contents daily appearing as soft faeces were estimated according to the following expression: [soft faeces excretion (g DM kg^{-1} BW d^{-1})/caecal contents (g DM kg^{-1} BW)] x 100. The values obtained were 117, 85, 72, 89 and 84% for lucerne hays A, B, C, D and E, respectively, indicating that the caecal contents for diet A tended to be removed more quickly from the caecum than for the others. Carabaño *et al* (1988) found that an increase of dietary fibre content from 24.6 to 43.9% NDF on DM implied a linear increase of caecal content appearing daily as soft faeces from 35 to 105%. The results of this study show that dietary NDF concentrations above 50% on DM might increase caecal retention time, as occurs with low fibre diets.

The lack of differences in feed intake and soft faeces excretion implied that the contribution of soft faeces to total DM intake was similar in all diets (15.1%, as average). These results agree with those reported by Gidenne and Lebas (1987) and Motta (1990), although there were large differences in dietary fibre content among the studies (47.9, 34.0 and 29.5% NDF on DM, on average, respectively).

The contribution of soft faeces to the total N intake averaged 20.5%. Rabbits fed lucerne hay A recycled daily through soft faeces 20 and 50% more total and microbial nitrogen than those fed lucerne E. The proportion of microbial to total nitrogen was also higher in lucerne A than in lucerne E (41 *vs* 32%). These results show a decrease of efficiency of microbial protein synthesis in the caecum when the lucerne fibre content increases. Caecal ammonia concentrations (> 11 mmol $N\text{-}NH_3$ l^{-1}) did not limit microbial protein synthesis for any of the diets studied, according to the minimal requirements of ammonia for ruminal microorganisms (3.6 mmol $N\text{-}NH_3$ l^{-1}; Roffler and Satter, 1975). However, a slower turnover rate has been related to a decrease of caecal microbial efficiency, because the supply of readily fermentable substrates is also reduced (Gidenne, 1992). The proportion of microbial nitrogen in the soft faeces and the caecal contents appearing daily as soft faeces were also significantly correlated in this study ($r = 0.61$, $P < 0.001$).

CONCLUSIONS

The type of lucerne hay affected significantly the caecal nitrogen metabolism. An increase in the lucerne NDF content decreased the caecal ammonia concentration and the amount of microbial nitrogen recycled daily through the soft faeces. However, no changes were found for the caecal parameters related to fibre fermentation (pH, molar proportion and total concentration of VFA in the caecum).

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